# FORMULATION AND EVALUATION OF LORNOXICAM NIOSOMAL TOPICAL GEL

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# CERTIFICATE

This is to certify that the dissertation entitled**, "FORMULATION AND EVALUATION OF LORNOXICAM NIOSOMAL TOPICAL GEL"** was done by **Mr. V. Palanivel** in the Department of Pharmaceutics, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmaceutics, is a bonafide work carried out by him, under the guidance and supervision of **Prof., Mr. A. Abdul Hasan Sathali**, **M.Pharm., (Ph.D).,** Professor and Head, in the Department of Pharmaceutics, during the academic year 2011 – 2012.

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I wish him success in all his endeavors.

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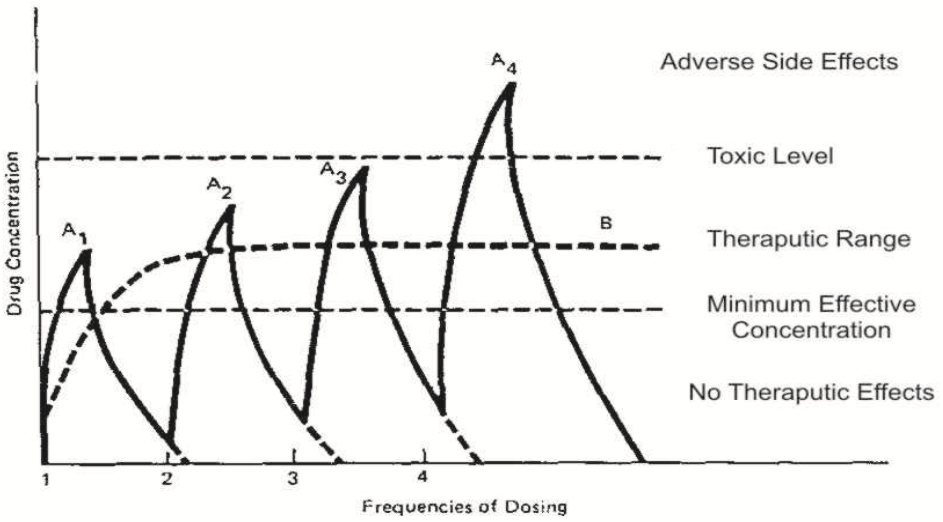
## INTRODUCTION

For many decades treatment of an acute disease (or) a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical conventional dosage forms (such as tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as drug carriers) **(**Chien Yie.W, 2005). Even today conventional drug delivery system occupies most of the part in a prescription as well as drug store. This type of drug delivery system is known to provide a prompt release of drug. Therefore, to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day (Chien Yie.W, 2005). This results in significant fluctuations in drug release.




Hypothetical drug concentration profiles in the systemic circulation resulting from the consecutive administration of multiple doses of an immediate-release drug delivery system ($A_1$, $A_2$, . . .) compared to the ideal drug concentration profile ($B$) required for treatment.

**FIGURE 1**

**DISADVANTAGES OF CONVENTIONAL DOSAGE FORM**

Poor patient compliance - increased chances of missing the dose of a drug with short half life for which frequent administration is necessary (Brahmankar D.M and Sunil B.Jaiswal, 2009).

A typical peak-valley plasma concentration-time profile is obtained which makes attainment of steady state condition is difficult.

The unavoidable fluctuations in the drug concentration may lead to under-medication or over-medication as the Css values fall or rise beyond the therapeutic range

The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever over-medication.

To overcome the above disadvantages, development of drug delivery systems capable of controlling the rate of drug delivery, sustaining the duration of therapeutic action and/or targeting the delivery of drug to a particular tissue (Chien Yie.W, 2005).

They are as follows (Aulton.M.E, 2002).

1. Delayed release.
2. Repeat action.
3. Prolonged release.
4. Sustained Release.
5. Extended release.
6. Controlled Release (Rate controlled).
7. Modified release.

**1) Delayed Release**

Delayed Release indicates the drug is not being released immediately following administration but at later time. Ex: enteric coated tablets; pulsatile release capsules.

**2) Repeat action**

Repeat action indicates that an indivual dose is released fairly soon after administration and second or third doses are subsequently at intermittent intervals.

**3) Prolonged release**

Prolonged release indicates that the drug is provided for absorption over a longer period of time than from a conventional dosage form. However there is an implication that onset is delayed because of an overall slower release rate from the dosage form.

**4) Sustained Release**

Sustained Release indicates an initial release of drug sufficient to provide a therapeutic dose soon after administration and then a gradual release over the extended period.

**5) Extended Release**

Sustained release dosage forms release drug slowly, so that plasma concentrations are maintained at a therapeutic level for a prolonged period of time. (Usually between 8 and 12 hours)

6) **Controlled Release**

Controlled release dosage forms release the drug at a constant rate, which is predictable and also the release rate is reproducible from one unit to another. It provides plasma concentrations that remain invariant with time.

**7) Modified Release**

Modified Release dosage forms are defined by the USP as those whose drug release characteristics of time course and for location are chosen to accomplish therapeutic or convenience objectives not offered by conventional forms whereas an extended release dosage form allows a 2 fold reduction in dosing frequency or increase in patient compliance or therapeutic performance. It is interesting to note that the USP considers that the terms controlled release prolonged release and sustained release are interchangeable with extended release.

**CLASSIFICATION OF CONTROLLED DRUG DELIVERY SYSTEMS (DDS)** (Brahmankar D.M and Sunil B.Jaiswal, 2009).

1. Rate-preprogrammed drug delivery systems.
2. Activation-modulated drug delivery systems.
3. Feedback-regulated drug delivery systems.
4. Site-targeting drug delivery systems.

**TARGETED or SITE-SPECIFIC DDS:**

Targeted DDS refers to systems that place the drug at or near the receptor site or site of action. Targeted drug delivery implies selective and effective localization of drug into the target(s) at therapeutic concentrations with limited access to target sites (Remington, 2002).

A targeted drug delivery system is preferred in the following situations:

Pharmaceutical: drug instability, low solubility.

Pharmacokinetic: short half-life, large volume of distribution, poor absorption.

Pharmacodynamic: low solubility, low therapeutic index.

Targeted drug delivery may provide maximum therapeutic activity by preventing drug degradation or inactivation during transit to the target sites. Meanwhile, it can protect the body from the adverse effects because of inappropriate disposition, and minimize toxicity of

potent drugs by reducing dose. An ideal targeted delivery system should be nontoxic, biocompatible, biodegradable and physic chemically stable *in vivo* and *in vitro.* The preparation of the delivery system must be reasonably simple, reproducible and cost-effective.

Site-targeted DDSs have also been characterized as

- Passive targeting: refers to natural or passive disposition of a drug-carrier based on the physiochemical characteristics of the system in relation to the body.
- Active targeting: refers to alteration of the natural disposition of the drug carrier, directing it to specific cells, tissues or organs; for e.g. use of legends or monoclonal antibodies which can target specific sites.
- Inverse targeting
- Legend mediated targeting
- Physical targeting (Triggered release)
- Dual targeting
- Double targeting
- Combination targeting *(Vyas S.P.and Khar R.K, 2002).*

Site-targeted DDS can be classified into three broad categories

First-order targeting: refers to DDS that delivers the drug to the capillary bed or the active site.

Second-order targeting: refers to DDS that delivers the drug to a specific cell type such as the tumor cells and not to the normal cells.

Third-order targeting: refers to DDS that delivers the drug intracellular.

Drug targeting often requires carriers for selective delivery and can serve following purposes-

- Protect the drug from degradation after administration.
- Improve transport or delivery of drug to cells.
- Decrease clearance of drug.

Combination of the above Carriers for drug targeting are of two types-

- Carriers covalently bonded to drug: where the drug release is required for pharmacological activity.
- Carriers not covalently bonded to drug: where simple uncoating of the drug is required for pharmacological activity. E.g. liposomes.

The various carriers used for drug targeting are-

- Polymeric carriers,
- Albumin,
- Lipoproteins,
- Liposomes,
- Niosomes,
- Microspheres,
- Nanoparticles,
- Antibodies,
- Cellular carriers and
- Macromolecules.

**COLLOIDAL DRUG CARRIERS:** (Vyas S.P and Roop K.Khar, 2008).

Colloidal drug delivery systems include micro- and Nanoparticles, macromolecular complexes (e.g. lipoproteins), liposomes and niosomes. In many cases, colloidal carriers are used to improve stability of the drug either in biological fluids or in the formulation, to develop extended-release systems with targeting features and/or to enhance the therapeutic

efficacy and reduce drug toxicity by modifying the distribution and controlling the disposition of the drug (Aulton .M.E).

**ADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM** (Jain N.K.2006).

- Employ less total drug, optimize therapy and improved patient compliance,
- Minimize (or) eliminate local side effects and drug accumulation with chronic dosing,
- Obtain less potential or reduction in drug activity with chronic use,
- Improve control of condition i.e.; reduce fluctuation in drug level, improve bioavailability and treatment efficiencies of some drugs,
- Make use of specific drugs e.g.; sustained release aspirin for morning relief of arthritis by dosing earlier,
- Maintenance of optimum therapeutic drug concentration in the blood with minimum fluctuations,
- Predictable and reproducible release rates for extended duration,
- Enhancement of activity duration for short half life drug,
- Elimination of frequent dosing, wastage of drug and inconvenience of night time administration of drug.
- Reduction of the incidence, degree of toxicity, side effects and irritation of GI tract caused by some orally administered drugs.

**DISADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM**

- High cost.
- Unpredictable (or) poor in-vitro – in-vivo correlation.
- Dose dumping.
- Reduce potential for dosage adjustment.

- Increases first pass clearance.
- Poor systemic availability in general (Vyas S.P.and Khar R.K, 2002).

**FACTORS INFLUENCING THE DESIGN AND PERFORMANCE OF CONTROLLED DRUG DELIVERY SYSTEMS**

**1) Drug Properties / Physiochemical Properties**

- Partition co-efficient.
- Drug Stability.
- Protein binding.
- Molecular size and diffusivity.
- Aqueous solubility (Vyas S.P.and Khar R.K, 2002) (Jain.N.K, 2006).

**2) Biological Properties**

- Absorption.
- Distribution.
- Metabolism.
- Elimination and biological half-life.
- Dose size.
- Route of drug delivery.
- Target sites.
- Acute or chronic therapy.
- The pathological disease.
- The patient condition.
- Duration of action.
- Margin of safety.
- Circadian rhythm.

**3) Physiological Properties**

- Prolonged drug absorption.
- Variability in GI Emptying and motility.
- Gastro Intestinal Blood flow.

**4) Pharmacokinetic Properties**

- Dose dumping.
- First Pass metabolism.
- Variability of urinary pH effect on drug elimination.
- Enzyme induction/inhibition upon multiple dosing.

**5) Pharmacological properties**

- Changes in drug effect upon multiple dosing.
- Sensitizing / tolerance.

**DRUGS UNSUITABLE FOR CONTROLLED DRUG DELIVERY**

1. Short / long elimination half life.
2. Narrow therapeutic index.
3. Poor absorption.
4. Active absorption.
5. Large doses.
6. Low aqueous s solubility.
7. Extensive first pass metabolism (Vyas S.P.and Khar R.K, 2002).

**FUTURE TRENDS IN CONTROLLED DRUG DELIVERY SYSTEM**

The most exciting and challenging opportunities in controlled drug delivery lie in the arena of responsive delivery system, with which it will be possible to deliver drug through implantable devices in response to a measured blood level and to deliver the drug precisely to a target size (Vyas S.P.and Khar R.K, 2002).

## CHAPTER-II

## VESICULAR SYSTEM-REVIEW

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain ampiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of ampiphilic building blocks. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies. Drug Carrier can be engineered to slowly degrade, react to stimuli and be site-specific. The ultimate Aim is to control degradation of drug and loss, prevention of harmful side effects and increase the Availability of the drug at the disease site. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces The toxicity if selective uptake can be achieved .Lipid vesicles are one type of many Experimental models of biomembranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of Vesicular drug delivery systems. Vesicular drug delivery system has some of the advantages like (Amit Kumar Jha *et al*., 2011).

Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.

Improves the bioavailability especially in the case of poorly soluble drugs.

Both hydrophilic and lipophilic drugs can be incorporated.

Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

These vesicular systems are accompanied with some problems like drug carriers and externally triggered (e.g., temperature, pH, or magnetic sensitive) carriers load drugs passively, which may lead to low drug loading efficiency and drug leakage in preparation, preservation and transport in vivo.

**Liposomes:**

The liposomes have emerged as most practically useful carriers for in-vivo drug delivery as majority of reports has concentrated on the use of phospholipids vesicles or liposomes as potential drug carrier systems. Liposomes or lipid based vesicles are microscopic (unilamellar ormultilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayer structures. The assembly into closed bilayer structures is a spontaneous process and usually needs some input of energy in the form of physical agitation, sonication, heat etc. Since lipid bilayer membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes. The lipid soluble or lipophilic drugs get entrapped within the bilayer membrane whereas water soluble or hydrophilic drugs get entrapped in the central aqueous core of the vesicles .Liposomes are potential carrier for controlled drug release of tumors therapeutic agents and antibiotic, for gene and antisense therapy through nucleic acid sequence delivery, immunization through antigen delivery and for anti-Parkinson's. In last one decade, pharmaceutical researchers use the tools of biophysics in evaluating liposomal dosage forms. Liposomes have covered predominantly medical, albeit some non-medical areas like bioreactors, catalysts, cosmetics and ecology.

**Advantage:**

- Liposomes supply both a lipophilic environment and aqueous "milieu interne" in one system and are therefore suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs and agents.
- Liposomes could encapsulate not only small molecules but also macromolecules like superoxide dismutase, hemoglobin, erythropoietin, interleukin-2 and interferon-g.
- Liposomes reduced toxicity and increased stability of entrapped drug via encapsulation
- (Eg.Amphotericin B, Taxol).
- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Alter the pharmacokinetic and pharmacodynamic property of drugs (reduced elimination,
- Increased circulation life time).

**Limitation:**

- High production cost
- Leakage and fusion of encapsulated drug / molecules.
- Sometimes phospholipids undergoes oxidation and hydrolysis
- Short half-life
- Low solubility
- Less stability.

**Niosomes:**

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the

nanometric scale. Although structurally similar to liposomes, they offer several advantages over them. Niosomes have recently been shown to greatly increase transdermal drug delivery and also can be used in targeted drug delivery, and thus increased study in these structures can provide new methods for drug delivery. In recent years, niosomes have been extensively studied for their potential to serve as a carrier for the delivery of drugs, antigens, hormones and other bioactive agents. Besides this, niosome have been used to solve the problem of insolubility, instability and rapid degradation of drugs.

**Advantages associated with Niosomes:**

- Niosomes are biodegradable, biocompatible and non immunogenic to the body.
- Niosomes can be utilized in the delivery of wide variety of drugs as it has capability to entrap hydrophilic, lipophilic as well as ampiphilic drugs.
- Niosomes shows controlled and sustained release of drugs due to depot formation
- Niosomes show a greater bioavailability than conventional dosage forms.
- Shape, size, composition, fluidity of niosomes drug can be controlled as and when required.
- Niosomes had been effectively used in targeting drugs to various organs.

**Limitation:**

Physical instability in niosomal dispersion during storage occurs due to vesicles aggregations, fusion and leaking. This may leads to hydrolysis of encapsulated drugs which affects the shelf life of the dispersion.

**Sphinosomes:**

Liposome stability problems are of course much more severe so it is very important task to improve the liposomal stability. Liposomal phospholipids can undergo chemical degradation

such as oxidation and hydrolysis either as a result of these changes or otherwise liposome maintained in aqueous suspension may aggregate, fuse, or leak their content. Hydrolysis of ester linkage will slow at pH value close to neutral. The hydrolysis may be avoided all together by use of lipid which contains ether or amide linkage instead of ester linkage (such are found in sphingolipid) orphospholipid derivatives with the 2- ester linkage replaced by carbomoyloxy function. Thus sphingolipid are been nowadays used for the preparation of stable liposomes known as Sphinosomes. Sphinosomes may be defined as "concentric, bilayer vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphinolipid.Sphinosomes are administered in many ways these include Parenteral route of administration such as intravenous, intramuscular, subcutaneous, and intra-arterial. Generally it will be administered intravenous or some cases by inhalation. Often it will be administered into a large central vein, such as the superior vena cava and inferior vena cava to allow highly concentrated solution to be administered into large volume and flow vessels. Sphinosomes may be administered orally or transdermal. In simple way we can say Sphinosomes is liposome which is composed of sphinolipid.

**Advantage:**

- Provide selective passive targeting to tumor tissue.
- Increase efficacy and therapeutic index.
- Increase stability via encapsulation.
- Reduction in toxicity of the encapsulated agent.
- Improve pharmacokinetic effect (increase circulation time).
- Flexibility to couple with site specific legends to achieve active targeting.

**Limitation:**

- Higher cost of sphingolipid hinders the preparation and use of these vesicular systems.
- Low entrapment efficacy.

**Pharmacosomes:**

Pharmacosomes bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicles. They are the colloidal dispersions of drugs covalently bound to lipids. Depending upon the chemical structure of the drug–lipid complex they may exist as ultrafine vesicular, micelles, or hexagonal aggregates. As the system is formed by linking a drug (pharmakon) to a carrier (soma), they are termed as Pharmacosomes. They are an effective tool to achieve desired therapeutic goals such as drug targeting and controlled release. The criterion for the development of the vesicular Pharmacosomes is dependent on surface and bulk interactions of lipids with drug. Any drug possessing an active hydrogen atom (- COOH, -OH, -NH2, etc.) can be esterifies to the lipid, with or without spacer chain that strongly result in an ampiphilic compound, which will facilitate membrane, tissue, or cell wall transfer, in the organism. The prodrug conjoins hydrophilic and lipophilic properties, thus acquires ampiphilic characters, and therefore found to reduce interfacial tension, and at higher concentrations exhibits geomorphic behavior.

**Advantage:**

- As drug is covalently bound, membrane fluidity has no effect on release rate, but in turn depends upon the phase-transition temperature of the drug-lipid complex.
- No leakage of drug take place as the drug is covalently linked to the carrier.
- Drug can be delivered directly to the site of infection.
- Drug release from Pharmacosomes is by hydrolysis (including enzymatic).

- Their degradation velocity into active drug molecule, after absorption depends very much on the size and functional groups of the drug molecule, the chain length of the lipids, and the spacer.
- Reduced cost of therapy.

**Limitation:**

- Synthesis of a compound depends upon its ampiphilic nature.
- Required surface and bulk interaction of lipids with drugs.
- Required covalent bonding to protect the leakage of drugs.
- Pharmacosomes, on storage, undergo fusion and aggregation, as well chemical hydrolysis.

**Transferosomes:**

Transferosomes was introduced for the effective transdermal delivery of number of low and high molecular weight drugs. Transferosomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayer properties. It consist of both hydrophilic and hydrophobic properties, high deformability gives better penetration of intact vesicles. These vesicular Transferosomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration. Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. There is provision for this, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of Transferosomes membrane is achieved by mixing suitable surface-active component sin the proper ratios. Transferosomes based formulations of local

anesthetics- lidocaine and tetracaine showed permeation equivalent to subcutaneous injections. Anti cancer drugs like methotrexate were tried for transdermal delivery using Transferosomes technology. This provided a new approach for treatment especially of skin cancer.

**Advantage:**

- Transferosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
- Transferosomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.
- Possess high entrapment efficiency, in case of lipophilic drug near to 90%.
- Used for both systemic as well as topical delivery of drug.

**Limitation:**

- Transferosomes are chemically unstable because of their predisposition to oxidative degradation.
- Purity of natural phospholipids is another criteria militating against adoption of Transferosomes as drug delivery vehicles.
- Transferosomes formulations are expensive.

**Future Perspective:**

Vesicular drug delivery systems are emerging with the diverse application in Pharmaceutics, Cosmetics and food industries. Their delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects. It also reduces the cost of therapy by imparting better biopharmaceutical properties to the drug, resulting in improved

bioavailability, especially in case of poorly soluble drugs. Now a day's various non-steroidal anti inflammatory drugs, proteins, cardiovascular, antineoplastic, antiglucoma, ant diabetic drugs that are incorporated with vesicular system are available in a commercial market that are playing a vital role to cure from a disease, hence improving the health of human kinds. Some of the emerging vesicular drug delivery system is listed below.

# CHAPTER III

## NIOSOMES-REVIEW

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerolether class and cholesterol with subsequent hydration in aqueous media. They are vesicular systems similar to liposomes that can be used as carriers of ampiphilic and lipophilic drugs. The method of preparation of niosome is based on liposome technology. The basic process of preparation is the same i.e. hydration by aqueous phase of the lipid phase which may be either a pure surfactant or a mixture of surfactant with cholesterol. After preparing niosomal dispersion, unentrapped drug is separated by dialysis centrifugation or gel filtration. A method of in-vitro release rate study includes the use of dialysis tubing. Niosomes are promising vehicle for drug delivery and being non-ionic; it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants. They are very similar to the liposomes. Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. Niosomes have shown promise in the release studies and serve as a better option for drug delivery system. This class of vesicles was introduced by Handjani – Vila *et al*. They are vesicular systems similar to liposomes that can be used as carriers of ampiphilic and lipophilic drugs (Thersa, 1998). One of the reasons for preparing niosomes is the assumed higher chemical stability of the surfactants than that of phospholipids, which are used in the preparation of liposomes. Due to the presence of ester bond, phospholipids are easily hydrolyzed (Breimer, 1985). Unreliable reproducibility arising from the use of lecithin's in liposomes leads to additional problems and has led scientist to search for vesicles prepared from other material, such as non-ionic

surfactants. Niosomes are promising vehicle for drug delivery and being non-ionic; it is less toxic and improves the therapeutic index of drug by restricting it suction to target cells.

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic

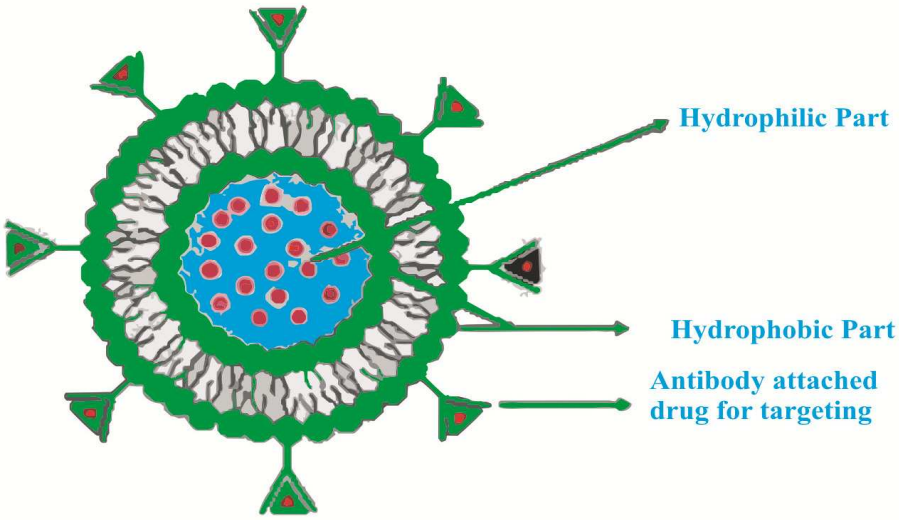



**FIGURE 2: Structure of niosome vesicle**

Surfactant of the alkyl or dialkyl polyglyceryl ether class and cholesterol with subsequehydration in aqueous media. (Malhotra) In niosomes, the vesicles forming ampiphilic is a non-ionic surfactant such as Span – 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate. The first report of non-ionic surfactant vesicles came from the cosmetic applications devised by L'Oreal (Buckton *et al*., 1995). The concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Niosomes represent a promising drug delivery module. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure

(Don *et al*., 1997).Niosomes are thoughts to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc. Various types of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parental, etc (Handjani *et al*., 1979; Pranshu Tangri *et al*., 2011).

**ADVANTAGES OF NIOSOMES**

- The niosomal drug delivery is a potential drug delivery method for controlled and targeted drug delivery; the major advantages of these vesicular drug carriers are;
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non aqueous phase.
- The vesicle suspension is water–based vehicle. This offers high patient compliance in comparison with oily dosage forms.
- They are osmotically active and stable, as well as they increase the stability of entrapped drug.
- Handling and storage of surfactants requires no special conditions.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They can be made to reach the site of action by oral, Parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.
- Niosomes possess an infrastructure consisting of hydrophilic, ampiphilic and lipophilic moieties to gather and as a result can accommodate drug molecules with a wide range of solubility's.
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume; surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

**FACTORS GOVERNING NIOSOME FORMATION:**

**Amount and type of surfactant**

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span85 (HLB 1.8) to Span20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant. Theoretically niosome formation requires the presence of a particular class of ampiphilic and aqueous solvent. In certain cases cholesterol is required in the formulation and vesicle aggregation for example may be prevented by the inclusion of molecules that stabilize the system against the formation of aggregates by repulsive stearic or electrostatic effects .The bilayer of the vesicles are either in the so called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the

presence of other components such as cholesterol. In the gel state, alkyl chains are present in a well ordered structure, and in the liquid state, the structure of the bilayer is more disordered. The surfactants and lipids are characterized by the gel liquid phase transition temperature (TC). Phase transition temperature also affects entrapment efficiency.eg:span 60 is the better surfactant because it is having high phase transition temperature and low HLB value so it form vesicle of good size without micelle formation (D.Akhilesh *et al.,* 2012).

**Cholesterol content and charge:**

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayer and on the other, cholesterol decreases the chain order of gel state bilayer. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase an increase in cholesterol content of the bilayer resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayer obtained. Presence of charge tends to increase the interlamellar distance between successive bilayer in multi lamellar vesicle structure and leads to greater overall entrapped volume. The level of surfactant/lipid used to make niosomal dispersions is generally 10-30 mM (1- 2.5% w/w).Altering the surfactant: water ratio during the hydration step may affect the system's microstructure and hence the system's properties. However increasing the surfactant/lipid level also increases the total amount of drug encapsulated, although highly viscous systems result, if the level of surfactant/lipid is too high.

**Nature of the encapsulated drug:**

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayer,

thereby increasing vesicle size. E.g.: DOX has been shown to alter the electrophoretic mobility of hexadecyldiglycerol ether (C16G2) niosomes in a pHdependent manner, an indication that the amphipathic drug is incorporated in the vesicle membrane.

**Structure of surfactants**

The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of Surfactants can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation,

$$\mathbf{CPP = V/LC \times a0}$$

Where,

CPP $\leq$ 0.5 micelles form

CPP = 0.5 – 1 spherical vesicles form

CPP = $\geq$ 1 inverted vesicles form

V = Hydrophobic group volume

Lc = the critical hydrophobic group length,,

$a_0$ = the area of hydrophilic head group.

Span 60 is the good surfactant because it has CPP value between 0.5 to 1

**Temperature of hydration**

Hydration temperature influences the shape and size of the noisome. The hydrating temperatures used to make niosomes should usually be above the gel to liquid phase transition temperature of the system.

**TYPES OF NIOSOMES**

They are divided in to three types

- Multilamellar niosomes (>0.05µm)
- Small unilamellar niosomes (0.025-0.05µm)
- Large unilamellar niosomes (>0.01µm)

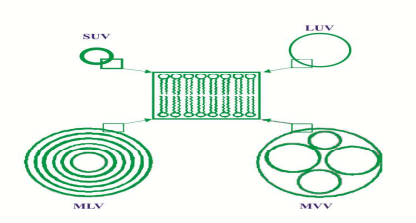



**Figure 3: Schematic illustration of different size and number of lamellae**

**SUV: Small unilamellar vesicles LUV: Large unilamellar vesicles**

**MLV: Multilamellar vesicles, MVV: Multi vesicular vesicles.**

**Method of preparation of niosomes**

Various methods are reported for the preparation of niosomes such as:

1. Ether injection method
2. Hand shaking method (Thin film hydration technique)
3. Sonication method
4. Reverse phase evaporation technique (REV)
5. Micro fluidization
6. Multiple membrane extrusion method
7. Trans membrane pH gradient (inside acidic) drug uptake process (remote loading)
8. Bubble method

9. Formation of niosomes from proniosome

***1. Ether injection method***

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether (volatile organic solvent) into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14- gauge needle into an aqueous solution of material. Vaporization of ether (volatile organic solvent) leads to formation of single layered vesicles. Depending upon the conditions used the diameter of the vesicle range from 50 to 1000 nm (Raj. K. Keservani *et al.,* 2011).

***2. Hand shaking method (Thin film hydration technique)***

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

***3. Sonication***

In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a Sonicator with a titanium probe to yield niosomes.

***4. Reverse phase evaporation technique (REV)***

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate

buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

***5. Micro fluidization***

It is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a smaller size, greater uniformity and better reproducibility of niosomes formed.

***6. Multiple membrane extrusion method***

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes, solution and the resultant suspension extruded through which are placed in series for up to 8 passages. Multiple membrane extrusion method is better for the controlling of niosome size.

***7. Trans membrane pH gradient (inside acidic) drug uptake process (remote loading)***

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes.

***8. Bubble method***

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

***9. Formation of niosomes from proniosome***

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "Proniosome".

**SEPARATION OF UNENTRAPPED DRUG**

1) **Dialysis:** The aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer or normal saline or glucose solution.

2) **Gel Filtration**: The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3) **Centrifugation:** The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug (Sudhamani.T. *et al*., 2010).

## CHARACTERISTATION OF NIOSOMES:

### Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug.

$$\textbf{Entrapment efficiency} = \frac{(\textbf{Amount of drug entrapped})}{(\textbf{Amount of total drug taken})} \boldsymbol{X\ 100}$$

### Vesicle diameter

Niosomes assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy and freeze fracture electron microscopy.

### In-vitro release

A method of in-vitro release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer is analyzed for the drug content by an appropriate assay method (Sakthivel M. *et al.,* 2012).

**Table No: 1 Analytical method for characterizing niosomes** (Rekha Rao *et al.,* 2011).

| S.NO. | PARAMETER(S) | METHOD(S) |
|---|---|---|
| 1. | Morphology | Transmission Electron Microscopy, Scanning Electron Microscopy, Optical Microscopy(OM), Cryo- Scanning Electron Microscopy, Freeze Fracture Microscopy. |
| 2. | Vesicle size determination and Size distribution | Dynamic Light Scattering using particle Size Analyzer(PSA), Malvern Master Sizer, Photon Correlation Spectroscopy (PCS), OM, SEM, Laser Diffraction PSA. |
| 3. | Zeta potential/ Surface Charge | Micro-electrophoresis meter, High Performance Capillary electrophoresis and Malvern Zeta Sizer (Zeta meter) |
| 4. | Rheological Properties (Elasticity) | Ostwald U-tube, Low shear Rheo Analyzer & Extrusion method. |
| 5. | Micro viscosity of niosomal membrane | Spectroflurophotometer |
| 6. | Viscosity | Ostwald's viscometer |
| 7. | Membrane micro-structure | Negative Staining TEM. |
| 8. | Lamellarity | OM, TEM |
| 9. | Bilayer spacing and thickness | X-Ray Scattering Analysis. |
| 10. | Gel-Liquid transition temperature & Thermal Analysis | Differential Scanning Calorimetry, Differential Thermal Analysis, & Hot Stage Microscopy. |
| 11. | Circular Dichroism | Spectropolarimeter. |
| 12. | Micro polarity measurement | Fluorescence Spectrophotometer. |
| 13. | Fluidity of vesicles | Differential Polarized Phase Fluorimetry |
| 14. | Turbidity measurement | UV-Visible Diode Array Spectrophotometer. |
| 15. | Entrapment Efficiency | Centrifugation method, Dialysis method, Gel Exclusion Chromatography. |
| 16. | In-vitro release rate | Using dialysis membrane. |
| 17. | Permeation study | Franz Diffusion Cell. |
| 18. | Conductivity | Conduct meter |

## TOXICITY AND STABILITY

Non-ionic surfactants used in niosomes are non-toxic and no toxic effects have been reported so far in animal studies due to the use of niosomes as drug carriers. Jain *et al* didn't observe any morphological changes on storage for three months. Baille *et al* determined the stability in buffer and reported that the amount of entrapped solute would be retained under long term storage conditions.

### Comparison of Niosome v/s Liposome

Niosomes are different from liposomes in that they offer certain advantages over liposomes. Liposomes face problems such as they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes do not have any of these problems. Also since niosomes are made of uncharged single-chain surfactant molecules as compared to the liposomes which are made from neutral or charged double chained phospholipids, the structure of niosomes is different from that of liposomes. However Niosomes are similar to liposomes in functionality. Niosomes also increase the bioavailability of the drug and reduce the clearance like liposomes. Niosomes can also be used for targeted drug delivery, similar to liposomes. As with liposomes, the properties of the niosomes depend both- on the composition of the bilayer, and the method of production used (Stuti Gupta *et al*., 2011).

# CHAPTER IV

# TOPICAL DRUG DELIVERY SYSTEM

Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders like acne or the cutaneous manifestations of a general disease like psoriasis with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use. The delivery of a drug to a specific site, topical formulations are probably among the most challenging products to develop. An effective topical formulation needs to provide a stable chemical environment in a suitable dispensing container in order to accommodate multiple compounds that may have different, if not incompatible, physicochemical characteristics. Once applied, a topical formulation must interact with the skin environment, which can influence the rate of the release of the compounds in order to achieve adequate skin absorption (Imran K. Tadwee *et al*., 2012).

The excipients themselves will exert additional physical effects on the skin, such as drying, occluding, or moisturizing. Research and technology have brought a better understanding of the physics, chemistry, pharmacodynamic, and pharmacokinetics for drugs used to treat acne. These insights have resulted in new delivery systems that are capable of enhancing the efficacy, tolerability, and cosmetic acceptability of topical formulations.

Topical drug delivery offers the advantages of ease of delivery, a cooperative patient, increased compliance as well as the avoidance of first-pass metabolism. Disadvantages are the lack of, or reduced rates of absorption and cosmetic considerations. New drug delivery technology and penetration enhancers may help to obviate some of these objections. There are important issues to consider as you contemplate development of a topical dermatological product. You may already have experience with oral or Parenteral products, but there are challenges and issues which are unique to development of topical formulations.

A topical formulation must be aesthetically pleasing, in addition to being both physically and chemically stable, and this may require numerous excipients. The formulation must allow for optimal penetration of the drug into the skin, a complex tissue. Skin pH is approximately 5.5; thus the pH of the formulation may change following application to the skin.

A successful topical dermatological formulation can be considered to be one that satisfies the target product profile and is 1)Physically and chemically stable having adequate shelf life, 2) Releases drug from the formulation and delivers it into the skin as required for the target indication, 3) Is cosmetically elegant and acceptable to patients, 4) Contains only excipients that are necessary,FDA approved or acceptable from a regulatory perspective, and acceptable for the disease state,5) Is easy to apply and compatible with the desired packaging, and 6) Can be manufactured with a process that is scalable to commercial levels. There are challenges during almost every development program. It is important to be able to anticipate problems, prevent them where possible, and to understand how to correct those that do occur.

**SKIN**

Skin is the largest organ of 1.5 to 2 $m^2$ in adult which covers the whole body. Thickness of skin varies from place to place i.e.; it is so thick in palm, foot and so thin in eyelid. (K.J.W.wilson and Anne lalaugh 1999,).

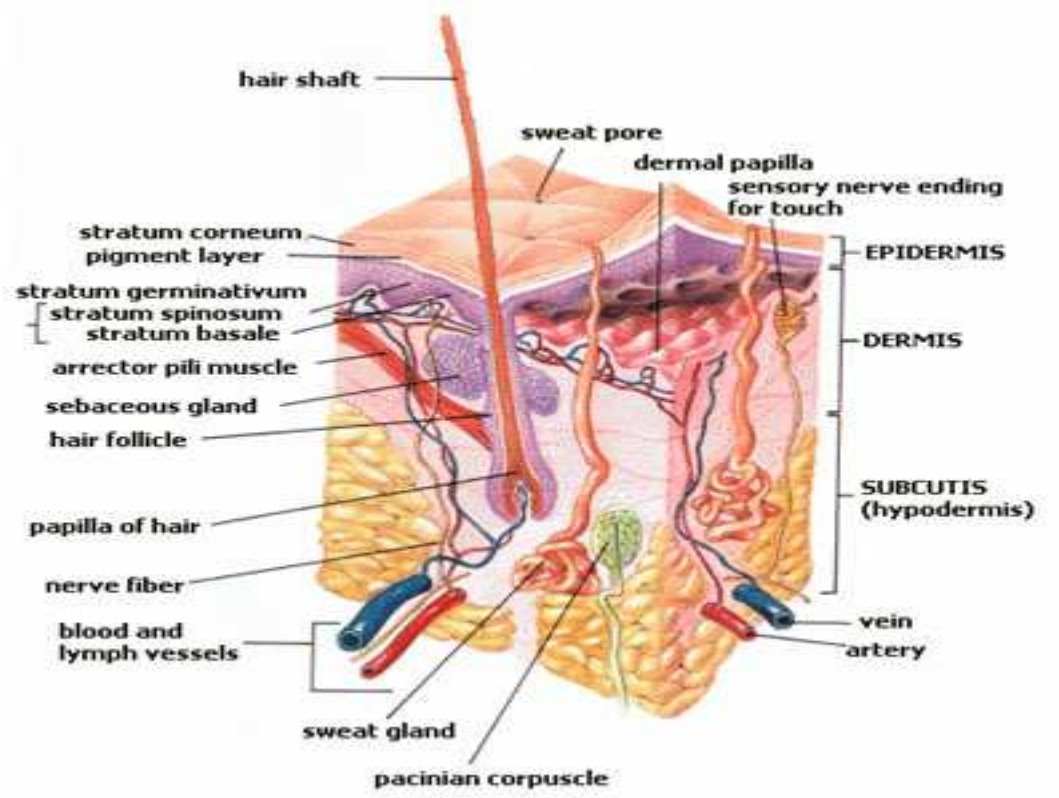



**FIGURE 4: Structure of skin**

The skin is broadly classified into two layers. They are;

A) Epidermis

B) Dermis.

**A. Epidermis**

It is the most superficial (or) outermost layer of skin. The cells in the epidermis shed periodically and replaced by new cells usually a complete replacement of epidermis takes about 40 days.

**Various Layers in Epidermis**

There are about four layers. They are;

1. Stratum corneum.
2. Stratum lucidum.
3. Stratum granulosm.
4. Germinative layer

**B. Dermis**

Dermis consists of the following things in it

1. Blood vessels.
2. Lymph vessels.
3. Sensory (somatic) nerve ending.
4. Sweat glands and their ducts.
5. Hair roots, hair follicles and hairs.
6. The arrectores pilorum – involuntary muscles attached to the hair follicles.
7. Sebaceous glands.

Hairs, secretions from sebaceous glands and ducts of sweat glands pass via the epidermis to reach the surface.

**FUNCTION OF SKIN**

It does major functions to the human body. They are;

- Mechanical function
- Protective function

Microbiological barrier

Chemical barrier

Radiation barrier

Electrical Barrier

- Regulation of body temperature
- Formation of vitamin D
- Sensation
- Absorption
- Excretion

## RATIONAL APPROACH TO DELIVER THE DRUG VIA SKIN

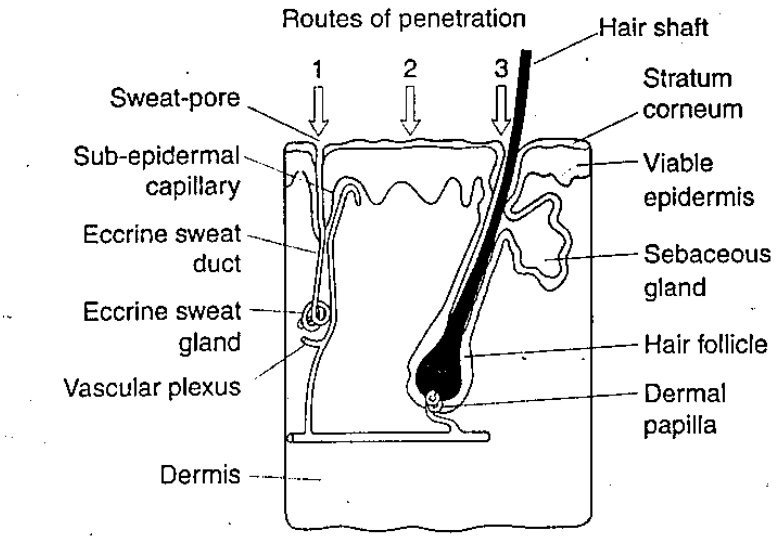
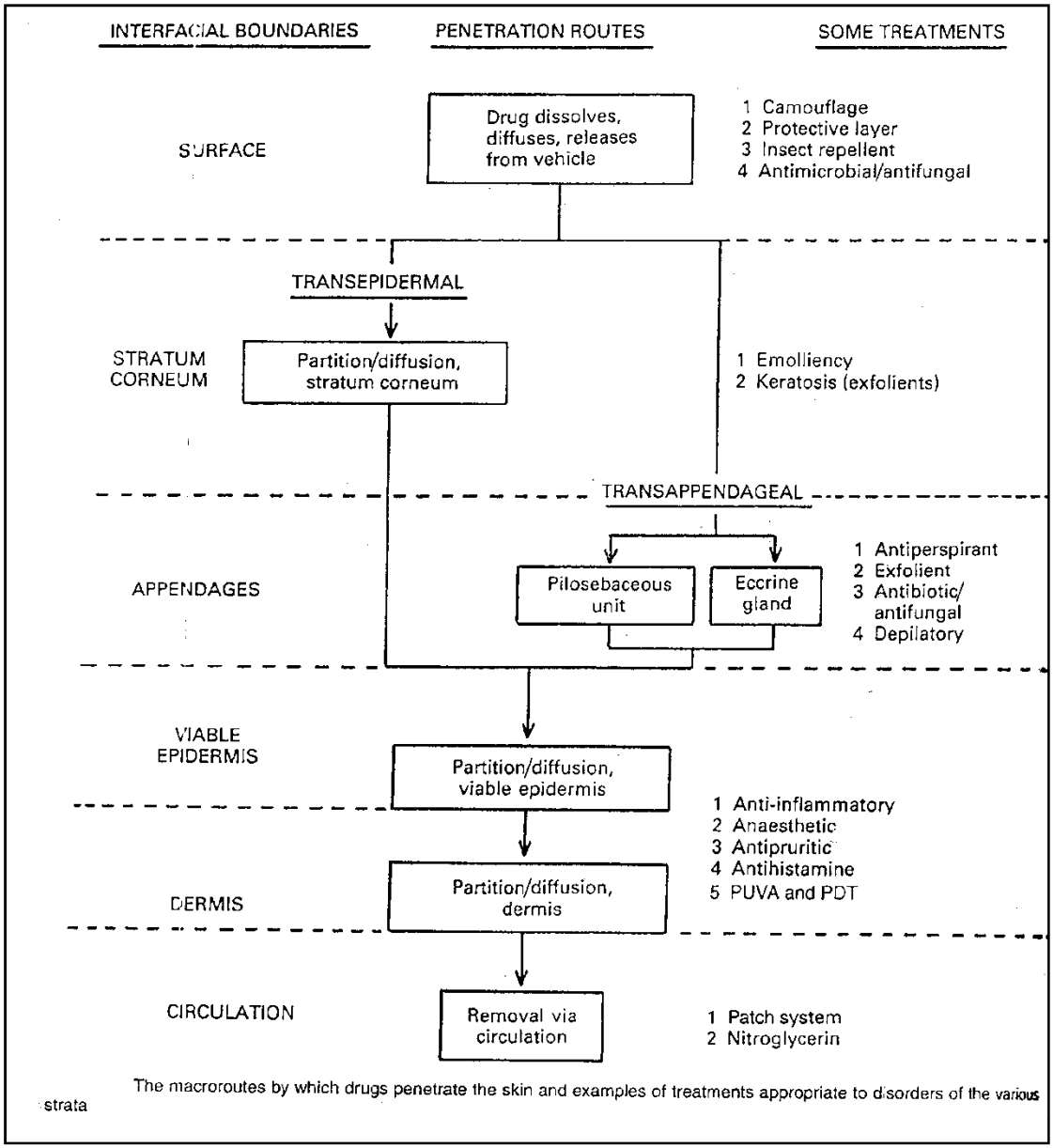



**FIGURE 5**

There are three main ways to approach the problem of formulating a successful topical dosage form. (M.E.Aulton, 2004).

- Manipulating the barrier function of the skin
- Directing drugs to the viable skin tissues without using oral, systemic (or) other routes.
- Using skin delivery for systemic treatment.

Dermatologist aim five main target regions. They are skin surface, horny layer, viable epidermis & upper dermis, skin glands and systemic circulation.




The macroroutes by which drugs penetrate the skin and examples of treatments appropriate to disorders of the various strata

**FIGURE 6**

## FACTORS AFFECTING DRUG DELIVERY

Transdermal route should have the capability to deliver the drug, regardless of size (or) structure at a predetermined rate. But there are some factors which influence the rate of drug delivery. There are two types of factors.

**A. Biological factors**

Skin condition.

Skin age.

Amount of blood flow.

Regional Skin sites.

Skin metabolism.

Species differences.

**B. Physiochemical Factors**

Skin hydration.

Temperature and pH

Diffusion Coefficient

Drug applying surface area.

Drug Concentration.

Partition Coefficient.

Molecular size and shape.

**TYPES OF TREATMENT ACHIEVED BY TOPICAL DRUG DELIVERY**

Camouflage.

Protection effects

Insect repellant.

Antimicrobial.

Antifungal.

Emolliency.

Keratosis.

Antiperspirant.

Exfolient.

Antibiotic.

Depilatory.

Anti inflammation.

Anti pruritic.

Local anesthetic.

PUFA and PDT.

Anti histamine.

Anti angina.

Anti-ischemic.

**VARIOUS TYPE OF DOSAGE FROM USED IN TOPICAL DRUG DELIVERY**

Liquid preparations.

Gels (jellies).

Powders.

Ointments.

Creams.

Paste.

Aerosols.

Poultice.

Transdermal patch.

**ROLE OF NIOSOMES IN TRANSDERMAL DRUG DELIVERY SYSTEM**

Niosomes can be used to deliver both hydrophobic and hydrophilic drugs via topical route. Although niosomes were tried for various routes it is used in the market for topical route . Studies showed that an enhanced delivery of drugs when encapsulated in niosomes. Niosomes increase skin penetration of drugs and it can act as local depot for sustained release

of dermal active compounds. When non ionic surfactants are incorporated into niosomes they are much better tolerated by the skin then when they are used in emulsion.

**NON-STERODIAL ANTI-INFLAMMATORY DRUGS**

Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs or NAIDs are drugs with analgesic and antipyretic effects and which have, in higher doses, anti-inflammatory effects. The term nonsteroidal is used to distinguish these drugs from steroids, which have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are usually in that they are non-narcotic. The most prominent members of this group of drugs are aspirin, ibuprofen and naproxen partly because they are available over the counter in many areas. (Tanu Bhargava et al., 2011).

**Mechanism of action:**

Most NSAIDs act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyzes the formation of prostaglandins and thromboxane form arachidonic acid. Prostaglandins act as messenger molecules in the process of inflammation .This mechanism of action was elucidated by John Vane (1927- 2004), who later received a Nobel Prize for this work. NSAIDs have antipyretic activity and can be used to treat fever. Fever is caused by elevated levels of prostaglandin E2, which alters the firing rate of neurons within the hypothalamus, that control thermoregulation. Antipyretics work by inhibiting the enzyme COX, which causes the general inhibition of prostanoid biosynthesis (PGE2) within the hypothalamus. PEG2 signals to the hypothalamus to increase the body's thermal set point. Ibuprofen has been shown to be more effective as an antipyretic than acetaminophen. Arachidonic acid is the precursor substrate for cyclooxygenase leading to the production of prostaglandins F, D and E.

**Classification:**

NSAIDs can be broadly classified based on their chemical structure;

1) **Prpoionic acid derivatives**

Ibuprofen

Naproxen

Ketoprofen

Flurbiprofen.

2) **Acetic acid derivatives**

Indomethacin

Diclofenac.

3) **Enolic acid derivatives**

Piroxicam

Meloxicam

Lornoxicam.

4) **Fenamic acid derivatives**

Mefenamic acid

Tolfenamic acid

5) **Selective COX-2 inhibitors**

Celecoxib

Valdecoxib

Etoricoxib

**USES:**

Rheumatoid arthritis

Osteoarthritis

Acute gout

Metastatic bone pain

Postoperative pain

Pyrexia

Renal colic

**ADVERSE EFFECTS:**

The two main adverse drug reaction associated with NSAIDs relate to gastrointestinal effects and renal effects of the agents. These effects are dose-dependent, and in many cases severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, and death limiting the use of NSAIDs therapy.

# CHAPTER-V

# LITERATURE REVIEW

**Vijay S. Jatav *et al*., 2010.** The niosomes containing rifampicin were prepared using various non ionic surfactants of sorbitan ester class and cholesterol in 50:60 percent mol fraction ratios for sustain release. To improve the dissolution rate of niosomes prepared by hand shaking method. The entrapment efficiency was decrease progressively span-85>span-80>span-60>span-40>span-20. The invitro release rate studies given span-20 maximum cumulative percent and span-85 minimum cumulative percent. The handshaking method is a simple and efficient technique for designing functional niosomes for hydrophobic or ampiphilic drugs.

**Lakshmi.P.K *et al*., 2009.** The niosomes containing salbutamol sulphate was prepared using span 60 as the surfactant. In this niosomes prepared by various techniques. The drug encapsulation efficiency varied from 62% to 87%.Transmembrane pH gradient method was found to be most satisfactory which released 78.4% of drug in 24h.Tissue distribution studies in albino rats and bio availability studies in rabbits were carried out.

**Anand Kumar.Y *et al*., 2011.** Aceclofenac is a drug with narrow therapeutic index and short biological half-life. This study was optimizing niosomal formulation of Aceclofenac in order to improve its bioavailability. The noisome formulation were evaluated particle size, entrapment efficiency and drug release were studied. The in vitro release studies used the dialysis membrane. The mechanism of drug release was governed by Peppas model.

**Ibrahim A. Alsarra *et al*., 2005.** Having a considerable ability to improve the permeability of drugs through lipid membranes, niosomes have been utilized as carriers to enhance atenolol absorption from the gastrointestinal tract. Two methods have been adopted to prepare niosomes, the proniosome-derived method (A) and the conventional film hydration method (B).Their morphology, vesicle size, drug encapsulation efficiency, in vitro release were compared to the two methods. High encapsulation efficiencies of 98.6% and 93.4% were achieved by methods A and B, respectively. In vitro drug release has been significantly retarded from both types of niosomes. The release kinetics non-Fickian behavior. Permeation through an everted intestinal sac showed a significant enhancement perfect for both types' niosomes.

**Vyas jigar *et al*., 2011** Erythromycin was entrapped into niosomes by thin film hydration technique and various process parameters were optimized by partial factorial design. Demonstrated that encapsulation of erythromycin into niosomal gel formulation improves skin retention which may be reflected, based on prior hypothesis, as significantly improved therapeutic response and considerably reduced adverse symptom. However, the role of niosomal erythromycin gel of this study can only be settled after clinical evaluation of the product with large number of patient with special focus on the adverse symptom of the therapy.

**Srikanth.K *et al*., 2010** Meloxicam entrapped niosomes were prepared by thin film hydration technique using Nonionic surfactants (span-80, span-60, Tween-80, Tween-60), cholesterol and drug in different ratios. To develop a topical Meloxicam niosomal gel using non-ionic surfactants to avoid the side effects developed when Meloxicam taken orally. The niosomal gel showed better pharmacological activity than the Meloxicam plain gel indicating a promising potential of the Meloxicam niosomal gel as an alternative to the conventional dosage forms.

**Fathy I.abd-allah *et al*., 2010** Piroxicam in micro emulsion formulations from different pharmaceutical topical preparation including different gel bases such as methyl cellulose, carbopol 934. The usage of the micro emulsion technique led to improvement in Piroxicam availability which can offer many promising features for its use as a topical vehicle for Piroxicam delivery. In vitro release, rheological properties and shelf life, the HPMC gel base containing 0.5% Piroxicam in micro emulsion formula was the best among the studied formulations.

**Saleem.M.A *et al.*, 2010** Solid dispersion complexes of Meloxicam were prepared by using cyclodextrins, PVP, and urea by kneading method in different molar and weight ratios. The solubility, dissolution and permeability was significantly enhanced by using solid dispersion of Meloxicam. Hence Meloxicam solid dispersion incorporated gel shows highest drug permeation through rat skin as compared

**Mahmoud Mokhtar *et a1*., 2008** studied the effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from Proniosomes. Proniosomal gels or solutions of flurbiprofen were developed based on span 20, span 40,span 60, and span 80 without and with cholesterol. Niosomes formed immediately upon hydrating proniosomal formulae. The entrapment efficiency (EE %) of flurbiprofen (a poorly soluble drug) was either determined by exhaustive dialysis of freshly prepared niosomes or centrifugation of freeze-thawed vesicles. The influence of different processing and formulation variables such as surfactant chain length, cholesterol content, drug concentration, total lipid concentration, negatively or positively charging lipids, and the pH of the dispersion medium on flurbiprofen EE% was demonstrated. Results indicated that the EE% followed the trend Sp 60

(C18)>Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18). Cholesterol increased or decreased the EE% depending on either the type of the surfactant or its concentration within the formulae.

**Toshimitsu Yoshioka *et al.*,1994** studied the formation of niosomes with a series of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85) using a mechanical shaking technique without sonication. 5(6)-Carboxyfhxxescein (CFI was used as a model solute to investigate entrapment efficiency and release. For Span 80, cholesterol and dicetyl phosphate DCP) in the molar ratio 47.5: 47.5: 5.0, entrapment efficiency increased linearly with increasing concentration of lipid. Entrapment efficiency increased with increasing cholesterol content when vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. Most efficient entrapment of CF occurred with Span 60 (HLB 4.7). Mean size of the un-sonicated niosomes showed a regular increase with increasing HLB from Span 85 (HLB 1.8) to Span 20 (HLB 8.6). The release rate of CF from vesicles depended on the surfactant used in the preparation of the vesicles.

**Manivannan Rangasamy *et al*., 2008** explained the acyclovir niosome preparation with different ratios of (1:1, 1:2, 1:3) cholesterol and Span 80 using hand shaking and ether injection process. The vesicles were in size range of 0.5-5 μm (hand shaking process) and 0.5-2.5 μm (ether injection process). The order of entrapment efficiency increases when Span 80 concentration was increased. *In vitro release* study & 16hrs for release.

**Abdul Hasan Sathali A. *et al*., 2010** developed terbinafine Hcl niosomes to the fungal affected cells for targeted delivery. Niosomes formulated by thin film hydration method using different ratios of non ionic surfactant (Tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The formulations evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. The formulation with

surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment. Niosomes tested for *in vitro* antifungal activity using the strain *Aspergillus niger* and compared with pure drug solution (as standard). The best (Tween 40 niosomes) formulation incorporated into gel bases and evaluated.

**Kandasamy Ruckmani *et al*., 2010** evaluated the effect of process-related variables like hydration time, sonication time, rotation speed of evaporation flask, changes in osmotic shock, viscosity, the effects of charge-inducing agent, centrifugation on entrapment and release from zidovudine niosomes. Formulation of zidovudine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. Non-sonicated niosomes were in the size range of 2-3.5 μm and sonicated niosomes had a mean diameter of 801 nm. Niosomes formulated with Tween 80 entrapped high amounts of drug and the addition of DCP enhanced drug release for a longer time (88.72% over 12 h). The mechanism of release was the Fickian type and obeyed first-order release kinetics.

**Tamizharasi S. *et al.,* 2009** formulated gliclazide-loaded niosomes and evaluated for their *in vitro* as well as *in vivo* characteristic in an attempt to improve the oral bioavailability. Microscopic observation confirmed that all particles were uniform in size and shape. The entrapment efficiency determined dialysis method. The in vitro release studies exhibited a prolonged drug release over a period of 24 h. The positive values of zeta potential indicated that the gliclazide niosomes were stabilized by electrostatic repulsive forces. The niosomes showing maximum entrapment and suitable release rate were selected for in vivo evaluation.

**Vijay Prakash Pandey *et al*., 2009** demonstrated the ofloxacin niosomes formation, to overcome ofloxacin eye drop solution drawbacks (poor bioavailability) characterization. Niosomes prepared by lipid film hydration method using span 60 and cholesterol (various molar ratios); Characterized for entrapment efficiency, in vitro drug release, surface charge, rheological character, physical stability, minimum inhibitory concentration, in vivo drug release and ocular irritation studies. The span 60: cholesterol in molar ratio of 100:60 showed higher entrapment of drug and released 73.77 % at 10th hr and the availability of drug in the aqueous humor was 4.373µg/ml ($C_{max}$), confirmed by HPLC method. The histopathology study also confirmed the safe use of niosomes.

**Manosroi.A *et al*., 2008** developed a novel elastic bilayer vesicle entrapped with NSAID, diclofenac diethyl ammonium (DCFD) for topical use. 18 formulations composing of DPPC or Tween 61 or Span 60 mixed with cholesterol and ethanol at 0–25% (v/v), by chloroform film method with sonication was developed. The elastic Tween 61 niosomes which gave no sedimentation, no layer separation, unchanged particle sizes (about 200 nm) were selected to entrap DCFD. Transdermal absorption through excised rat skin was performed by vertical Franz diffusion cell at 32±2 ◦C for 6 h. The *in vivo* anti-inflammatory activity was evaluated by ethyl phenylpropiolate (EPP)-induced rat ear edema ($n$ = 3).

**Prabagar Balakrishnan *et al*., 2009** reported to improve the low skin penetration and bioavailability characteristics shown by topical vehicle for minoxidil. Niosomes were prepared with thin film hydration method using Brij, Span and cholesterol at various ratios. The prepared systems were characterized for entrapment efficiency, particle size, zeta potential and stability. Skin permeation studies were performed using static vertical diffusion Franz cells & hairless

mouse skin. Higher entrapment efficiency was obtained with the niosomes prepared from Span 60, cholesterol at 1:1 molar ratio.

**Naresh Ahuja *et al*., 2008** prepared the niosomes containing lansoprazole (antacid and anti ulcer agent) by using reverse phase evaporation method. Non-ionic surfactant Span 60 was use to prepare the formulations. Niosomes are characterized for its entrapment efficiency, size range and invitro release of drug. For release study the phosphate buffered saline pH 8.6 was used and the samples were assayed by UV.

**Udupa N.,and Chandraprakash K.S ., 1990** examined the methotrexate (MTX) niosomes by preparing it using the thin film hydration method with Tween 80, 60, 40, Span 60, 40 and 20. The MTX- entrapped niosomes were separated from the unentrapped by dialysis. Measurement of niosome size was made by using a microscope with a mean diameter 4.5µm. The entrapment efficiency has also been observed to be greater for Span 60 and least for Tween 80 containing niosomes. The reason may be attributable to the increased lipophilicity of Span 60. The order of entrapment efficiciency increase as the lipophilicity increased.

**Pavala Rani .N. *et al*., 2010** studied that niosomes are vesicles mainly consisting of non-ionic surfactants that encloses and encompasses the drug molecules. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The prepared rifampicin and gatifloxacin niosomes showed a vesicle size in the range of 100-300nm, the entrapment efficiency were 73% and 70% respectively. The *invitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index.

**Sambhakar S *et al.*, 2011 prepared** niosomes containing cefuroxime axetil was prepared by film formation method by Span 40, 60 and 80 to overcome the bioavailability problem (25%). It is characterized by SEM for particle size and morphology. Entrapment efficiency and release study was carried out by dialysis. In-vitro absorption study was carried out by everted sac method and also the stability study of niosomes in presence of bile salts was determined. The vesicle size was found to be less than 5 μm and its polydispersity index was very low. Entrapment efficiency was found as Span 60 > Span 40 > Span 80. The in-vitro-release study indicated the controlled release profile of niosomes.

**Cosco D. *et al.*, 2009** evaluated niosomes made up of bola, Span 80 & cholesterol (2:5:2 molar ratio) are proposed as suitable delivery systems for the administration of 5-fluorouracil (5-FU), an antitumoral compound largely used in the treatment of breast cancer. The bola-niosomes, after sonication procedure, showed mean sizes of ~200 nm and a loading capacity of ~40% with respect to the amount of 5-FU added during the preparation. Similar findings were achieved with PEG-coated bola-niosomes. 5-FU-loaded PEG-coated and uncoated bola niosomes were tested on MCF-7 and T47D cells. Both bola-niosome formulations provided an increase in the cytotoxic effect. Confocal laser scanning microscopy studies were carried out to evaluate both the extent and the time-dependent bola-niosome-cell interaction. In vivo experiments on MCF-7 xenograft tumor SCID mice models showed a more effective antitumoral activity of the PEGylated niosomal 5-FU at a concentration ten times lower (8 mg/kg) than that of the free solution of the drug (80 mg/kg) after a treatment of 30 days.

**Ghada Abdelbary *et al*., 2008** investigated the feasibility of using niosomes as carriers for the ophthalmic controlled delivery of gentamicin sulphate. Niosomes prepared using various surfactants (Tween 60, Tween 80 or Brij 35), cholesterol and a negative charge inducer DCP in different molar ratios by thin film hydration technique. The entrapment efficiency determined by centrifugation. Photomicroscope, TEM and particle size analysis used to study the morphology and size of niosomes. Ocular irritancy test performed on albino rabbits, showed no sign of irritation for all tested niosomal formulations.

**Pratap S. Jadon *et al*., 2009** developed griseofulvin niosomes to improve its poor and variable oral bioavailability. Niosomes were prepared by using span 20, span 40, and span 60. The formulations prepared by thin film method and ether injection method. The influence of different formulation variables such as surfactant type, surfactant concentration, and cholesterol concentration was optimized for size distribution and entrapment efficiency for both methods. Higher entrapment efficiency obtained with span 60 niosomes prepared by thin film method. The niosomal formulation exhibited significantly retarded in vitro release as compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability, AUC of griseofulvin.

**Ismail A. Attia *et al.*, 2007** demonstrated the preparation of acyclovir niosomes in a trial to improve its poor and variable oral bioavailability. The niosomes were prepared by the conventional thin film hydration method. The % entrapment was found to be ~11%. The vesicles have an average size of 0.95 μm and a size range of 0.4 to 2.2 μm. Most of the niosomes have unilamellar spherical shape. The niosomal formulation exhibited significantly retarded release compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability (more than 2-fold increase) of acyclovir in rabbits. The

niosomal dispersion showed significant increase in the MRT of acyclovir reflecting sustained release characteristics.

**Mahmoud Mokhtar Ahmed Ibrahim *et al*., 2008** formulated and evaluated proniosomal transdermal carrier systems for flurbiprofen. Proniosomes were prepared using various non-ionic surfactants, namely span 20, span 40, span 60 and span 80 without and with cholesterol at percentages ranging from 0% to 50%. The effect of surfactant type and cholesterol content on drug release was investigated. Drug release was tested by diffusion through cellophane membrane and rabbit skin; rabbit skin showed lower drug diffusion rates compared to cellophane membrane. Drug release studies showed the proniosomal composition controlled drug diffusion rates to be either faster or slower than the prepared flurbiprofen suspensions in HPMC gels or distilled water, respectively. Microscopic observations showed that either proniosomal solutions or gel formulations immediately converted to niosomal dispersions upon hydration.

**Aranya Manosroi *et al*., 2010** studied the vesicles prepared with hydrated mixture of various non-ionic surfactants and cholesterol. The bilayer formation was characterized by X-cross formation under light polarization microscope. Membrane rigidity was measured by means of mobility of fluorescence probe as a function of temperatures. The stearyl chain (C18) non-ionic surfactant vesicles showed higher entrapment efficiency than the lauryl chain (C12) non-ionic surfactant vesicles. Cholesterol was used to complete the hydrophobic moiety of single alkyl chain non-ionic surfactants for vesicle formation.

**Ijeoma F. Uchegbu *et al*., 1998** summarized the achievements in the niosomes field. A number of groups worldwide have studied non-ionic surfactant vesicles (niosomes) with a view to evaluating their potential as drug carriers. Niosomes may be formed form a diverse array of ampiphilic. The self assembly of surfactants into niosomes is governed by the nature of the surfactant, the presence of membrane additives, the nature of the drug encapsulated and the actual method of preparation. The influence of formulation factors on niosome stability is also examined. Niosomes have been evaluated as immunological adjuvant, anti-cancer: anti-infective drug targeting agents, carriers of anti-inflammatory drugs, in diagnostic imaging, achieve transdermal and ophthalmic drug delivery

**Ajay B. Solanki *et al*., 2010** optimized the composition of niosomes containing Aceclofenac for transdermal application, with a view to improve permeation of drug during an extended period of time. Niosomes were prepared by thin film hydration technique. A $3^2$ factorial design was utilized to study the effect of the molar ratio of drug to lipid ($X1$) and volume of hydration medium ($X2$) on percentage drug entrapment (PDE) and vesicle size. Selected batches of niosomes were incorporated in to carbopol gel matrix to prepare the niosomal gel formulations, which were evaluated for *in vitro* release, skin permeation and *in vivo* studies. It was evident from the derived polynomial equations and constructed contour plot, a decrease in the level of $X1$ and an increase in the $X2$ lead to an increase in PDE and decrease in vesicle size. The polynomial equations and contour plot predicted the levels of independent variables $X1$ and $X2$ (0.19 and 0.46 respectively), for maximized response of PDE with constraints on vesicle size.

**Toshimitsu Yoshioka *et al.*, 1994** described in which niosomes are dispersed in an aqueous phase which is then emulsified in a non-aqueous continuous phase. The resultant vesicle (niosome) in water-in-oil (v/w/o) system allows the delivery of vesicles in a non-aqueous vehicle. The non-ionic surfactants used to prepare the vesicles (niosomes) are also employed in the emulsification step to minimize surfactant redistribution. The invitro release rate of CF showed a decrease in the order free solution >vesicle suspension >w/o emulsion >v/w/o emulsion. The release rate of CF from the v/w/o system depends on the nature of the surfactants used.

**Wei Hua *et al.*, 2007** prepared highly stable innocuous niosome composed of only three components in Span 80/PEG 400/H2O system. The niosome properties are studied by some means of freeze fracture replication-transmission electron microscopy, negative staining-transmission electron microscopy, dynamic light scattering and differential scanning Calorimetry. The obtained results indicate that the niosome can be stable for over one year. The niosome diameter is between 100 and 180 nm. The compositions of the system affect the preparation and properties of the niosome.

**Behrooz Nasseri *et al.*, 2005** explained the effect of cholesterol and temperature on the elastic properties of niosomal membranes. The mechanical characteristics of non-ionic bilayer membranes composed of span 60, cholesterol and poly-24- oxyethylene cholesteryl were studied by measuring the modulus of surface elasticity ($\mu$), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol and displayed a maximum around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease in $\mu$. Generally, the interaction of cholesterol with the span 60 should increase the

rigidity of the membrane. However, the latter effect may be due to the formation of *cholesterol clusters* at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayer compromising their uniformity, strength and permeability.

**Prasun Bandyopadhyay *et al*., 2007** studied of the self-organization of nonionic surfactant span 60 in presence of fatty alcohol (stearyl, cetyl and lauryl) is presented. When ethanol solution of the surfactant–fatty alcohol (1:1) mixture is added in water spontaneous large unilamellar vesicles (LUV) are formed. Vesicular suspension has been characterized by transmission electron microscopy, dynamic light scattering, and confocal laser scanning microscopy, dye entrapment and release studies.

**Chandra. A *et al*., 2008** Piroxicam are a widely used potent non-steroidal anti-inflammatory drug, with due potential for dermal delivery. Permeation of Piroxicam from proniosome based reservoir type transdermal gel formulation across excised rat abdominal skin was investigated using Keshery Chein diffusion cell. The lipid vesicles were evaluated for entrapment efficiency and vesicle size of niosomes formed. It was observed that Span 60 based formulations produced vesicles of smallest size and higher entrapment efficiency while those of Span80 produced vesicles of least entrapment efficiency. Incorporation of lecithin further enhanced entrapment efficiency. Proniosome were prepared by conventional technique and employing maltodextrin and sorbitol as base. The morphology of the proniosome was studied by scanning electron microscopy. Anti-inflammatory studies revealed that proniosome based transdermal drug delivery system of Piroxicam were promising carriers for delivery of Piroxicam. There was significant reduction in carrageenam induced rat paw inflammation compared to control.

**Abdul Hasan Sathali.A *et al*., 2011.** The aim of the present study was to formulate topical gel containing clobetasol propionate niosomes to prolong the duration of action and prevent its side effects. The clobetasol propionate niosomes were prepared by altering the ratios between various non-ionic surfactants (Span 40, 60, 80) and cholesterol by three methods such as thin film hydration method, ether injection method and hand shaking method. The prepared niosomes were subjected to drug content analysis, Entrapment efficiency, size analysis and *invitro* drug release studies. The higher entrapment efficiency (91.37%) was obtained with Span 60 niosomes (ratio of surfactant, cholesterol 1: 0.5) prepared by thin film hydration method was evaluated for its stability and formulated as gel formulation. The prepared niosomal gel (G2) and marketed gel (G3) were subjected to drug content analysis, *in vitro* drug release studies and *in vivo* pharmacodynamic studies. The results suggested that the niosomal delivery of clobetasol propionate in carbopol gel base acts as a suitable topical drug delivery system to prolong the duration of action.

**Chawda Himmat Singh *et al*., 2011** The niosomes provides several important advantages over conventional drug therapy. The main objective of this study was to design suitable niosome encapsulated drug delivery for anti-inflammatory drugs like nimesulide and evaluate the vesicle size, encapsulation efficiency, in vitro release and physical stability of the system. Non-ionic surfactants used were span 20, 40, 60 and cholesterol was used in different molar ratios. The niosomes prepared by lipid film hydration method were multilamellar vesicles (MLVS) and niosomes prepared by ether injection technique were unilamellar vesicles (ULVS) oroligolamellar vesicles. The higher entrapment efficiency was observed with MLVS prepared from span 60 and cholesterol in an 80:70 molar ratio. The *in vitro* diffusion study suggests that higher entrapment efficiency was related with slow release comparatively. The release pattern

shown by these formulations were zero order & Higuchi diffusion controlled mechanism. The physical stability study show that niosomal preparation stored at refrigerated temperature for 60 days show maximum drug retained for all the formulation compare to room temperature and elevated temperature conditions. Finding of all this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate after niosomal encapsulation of nimesulide.

**Biswal.S *et al.*, 2008** Vesicles prepared from self-assembly of hydrated non-ionic surfactants molecules are called niosomes. Niosomes exhibit more chemical stability than liposomes as non-ionic surfactants are more stable than phospholipids. Non-ionic surfactants used in formation of niosomes are polyglyceryl alkyl ether, glucosyldialkyl ether, crown ether, polyoxyethylenealkyl ether, ester-linked surfactants, and steroid-linked surfactants and a spans, and tweens series. Niosomes preparation is affected by processes variables, nature of surfactants, and presence of membrane additives and nature of drug to be encapsulated. This review article presents an overview of theoretical concept of factors affecting niosome formation, techniques of niosome preparation, characterization of niosome, applications, limitations and market status of such delivery system

**Lakshmi.P.K *et al*., 2011** present study topical niosomal gel in chitosan were prepared using urea as a model drug. The urea niosomes were prepared by both lipid layer hydration and transmembrane pH gradient method. Niosomes were prepared and characterized for various physical characters. Surfactants such as spans were used with cholesterol in 1:1 molar ratio with 5% dicetyl phosphate (DCP). The human volunteer study to test the irritancy was performed by human repeated insult patch test (HRIPT) test. PASI scoring was used to determine the severity

of the lesion. Niosomes prepared using span 60 showed a better entrapment than other spans. Both the niosomes showed uniform particle size distribution. SEM analysis showed smooth outer surface. A short-term stability studies showed that niosomal gel had better stability followed by niosomes prepared using transmembrane method and lipid layer hydration method. Permeation study of niosomal gel through the human skin showed better diffusion of drug through the skin and skin deposition study showed that better deposition of drug in comparison to plain gel. The niosomal urea gel and plain gel did not produce any irritation of the human skin. The gels were tested on psoriasis patients with less than 25% severity of any category of psoriasis. The niosomal gel produced significant reduction in the lesion ($p<0.05$) than plain urea gel. The niosomal urea gel produced greater reduction in total score and desquamation score compared to erythematic and infiltration score and proved that niosomal urea in chitosan gel can be used as an adjuvant in the treatment of psoriasis.

**Kumar *et al*., 2012** The purpose of this research is to design proniosomal gel drug delivery system of flurbiprofen in a trial to overcome the adverse effects associated with oral administration of the drug. This can be overcome by the use of vesicular drug delivery system. The potential of proniosome as a transdermal drug delivery system of flurbiprofen was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 4 weeks. The method of proniosome loading resulted in an encapsulation yield of 30.6 – 75.4%. *In-vitro* studies showed prolonged release of entrapped flurbiprofen. At refrigerated conditions, higher drug retention was observed. It is

evident from this study that proniosome are a promising prolonged delivery system for flurbiprofen and have reasonably good stability characteristics.

**El-Nahas.M *et al*., 2008** For topical administration of Meloxicam, micro emulsion gels and lip gels containing either ethyl oleate or oleic acid as an oil phase was prepared. In addition, Hydro gel and hydro alcoholic gels containing carbopol 940 as a gelling agent were also prepared. In vitro drug release through cellophane membrane and permeation through the excised rabbit skin in Sorensen's phosphate buffer (pH 7.4) containing 1% w/v sodium lauryl sulphate were performed .The influence of initial drug concentration was studied. The permeation property of Meloxicam from ethyl oleate micro emulsion which is the best formula achieved was studied in comparison to the commercially available Piroxicam gel. Moreover, the anti-inflammatory activity of Meloxicam, after oral and topical administration in rats was studied and compared to that of Piroxicam gel. The results of an in vitro drug release and its percutaneous permeation revealed that the ethyloleate micro emulsion gel showed the highest Results. Meloxicam gel (ethyl oleate micro emulsion gel 1%) showed good protection against inflammation as compared to FeldeneR gel in rats.

**Mettu srikanth reddy *et al*., 2011** Topical gels of Valdecoxib were prepared using different gelling agents (carbopol, HPMC, sodium alginate, sodium CMC). Formulations were evaluated for pH, rheological behavior, and drug content and *in-vitro* drug diffusion. Selected formulations of all the gelling agents were appeared to be non-Newtonian and showed pseudo plastic behavior. Drug content was high (>98%) in gels. Drug release from the carbopol gels increased with the increase in the concentration of propylene glycol up to 10%. However, drug release decreased as the concentration of the propylene glycol increased to 20%. The drug release

increased with the increase in concentration of ethanol. In case of gels containing HPMC, sodium alginate, sodium CMC as gelling agents, addition of Propylene glycol up to 5%, increased the release of drug from the gels. However, release decreased with increase in the concentration of Propylene glycol up to 10%. In case of HPMC gels, addition of ethanol decreased the release of Valdecoxib from the gels.

**Japan Patel *et al.*, 2011** Aceclofenac, a non-steroidal anti-inflammatory drug, has been used in the treatment of rheumatoid arthritis and osteoarthritis. In order to decrease the gastric ulcerogenic effects, Aceclofenac gels have been developed. This study was conducted to develop a gel formulation of Aceclofenac using gelling agents: carbopol, hydroxypropylmethylcellulose, carboxymethylcellulose sodium and sodium alginate. Effect of penetration enhancer on the release has been studied. The gels were evaluated for physical appearance, rheological behavior, drug release and stability. The drug release from all gelling agents through a standard cellophane membrane was evaluated using Keshery-Chien diffusion cell. All gels showed acceptable physical properties concerning color, homogeneity, consistency, spread ability and pH value. Among all the gel formulations, carbopol showed superior drug release than followed by Na CMC, HPMC and sodium alginate. Drug release decreased with increase in polymer concentration. Drug release was not linearly proportional with the concentration of penetration enhancer or co-solvents. Stability studies show that the physical appearance, rheological properties, and drug release remained unchanged upon storage for two months at ambient conditions.

**Vyas Jigar *et al.*, 2010** Niosomes, a non ionic surfactant vesicular formulation, have been explored extensively for topical application to enhance skin penetration as well as to improve

skin retention of drugs. In the present study, Benzyl peroxide was entrapped into niosomes by thin film hydration technique and various process parameters were optimized by partial factorial design. The optimized niosomal formulation was incorporated into HPMC K15 gel and extensively characterized for Percentage Drug Entrapment and in-vitro release performance. The stability of above formulation was studied at different temperatures. The present study demonstrated prolongation of drug release, increased drug retention into skin and improved permeation across the skin after encapsulation of Benzyl peroxide into niosomal topical gel.

**Chandira.R.M *et al*., 2010** In the present study, Adapalene gels were prepared using CMC Na, HPMC, HPC, Carbomer and combinations of cellulose derivatives; as base and PluronicPE-6200 as penetration enhancer for the treatment of Acne. The gels were evaluated for drug content, viscosity determination, in vitro permeation and stability studies. The drug content of the gels was found to range from 98-105.7 %. The viscosity of the gels ranged between 7100-83144 cps. In-vitro diffusion profile of Adapalene gel obtained in ethanol with water (80:20) indicates that 40.33% drug release found within 6 hrs. While 35.22% of marketed preparation. Although the difference is insignificant, the percentage release of drug was found to increase in the following orderofthepolymercomposition:

Carbopol980 > Carbopol940 > Carbopol934 > HPC > SODIUMCMC > HPC+SODIUM CMC > METHYL CELLULOSE > HPMC > HPC+HPMC. The best formulation was found to be stable at accelerated stability condition.

**Sureewan Duangjit *et al*., 2010** The goal of this study was developed and evaluate the potential use of liposome and Transferosomes vesicles in the transdermal drug delivery of Meloxicam, Meloxicam-loaded vesicles were prepared and evaluated for particle size, zeta potential, entrapment efficiency, loading efficiency, stability, and *in vitro* skin permeation. The vesicles

were spherical in structure, 90 to 140nm in size, and negatively charged (−23 to −43mV). The % Entrapment efficiency of Meloxicam in the vesicles ranged from 40 to 70%. Transferosomes provided a significantly higher skin permeation of Meloxicam compared to liposomes. Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry analysis indicated that the application of Transferosomes significantly disrupted the stratum corneum lipid. These researches suggest that Meloxicam-loaded Transferosomes can be potentially used as a transdermal drug delivery system.

# CHAPTER-VI

## AIM AND OBJECTIVE OF THE WORK

In this world peoples are affected pain problems. Normally they are taken pain killer tablets. Those types of tablets produce the intestinal problems. So, I will overcome that problems going for niosomal topical delivery.

Lornoxicam is a non-steroidal anti-inflammatory agent (NSAIDs), has been widely used in the treatment of rheumatoid arthritis, osteoarthritis. When it is taken orally it causes side effects such as nausea, drowsiness, diarrhea, fast heart beating, ringing in ears, etc. Because of these side effects the patient compliance may reduce. The best alternative route for administration of Lornoxicam is topical route.

However, even though Non-steroidal anti inflammatory drugs are used in the vast skin disorders and some side effects. The Lornoxicam main side effect was gastrointestinal bleeding and also low half life, poor solubility and high first pass metabolism.

To achieve the above aim, niosome is one of the right choices. Since they can entrap both hydrophilic and lipophilic drugs, it can be employed as a suitable drug carrier for Non-steroidal anti inflammatory drugs.

Then various Lornoxicam niosomes were prepared by altering the ratios between various non ionic surfactants and same amount of cholesterol by thin film hydration method.

The prepared niosomes were subjected to drug content analysis, Entrapment efficiency, Invitro drug release studies and Release kinetics of the formulations. The best niosomal preparations were formulated as gel. The niosomal gel was evaluated drug content, pH, viscosity, transmission electron microscopy. Finally the niosomal gel and plain gel were subjected to invitro drug release studies and in vivo animal studies.

## CHAPTER-VII

## PLAN OF WORK

The plan of work involves the following steps:

**1) STANDARD CURVE FOR LORNOXICAM**

Preparation of calibration medium

Determination of absorption maximum ($\lambda_{max}$) by UV spectrum

Calibration curve for Lornoxicam

**2) DRUG –EXCIPIENTS INTERACTION STUDIES**

Fourier Transform-Infrared Spectroscopic Studies (FT-IR)

Differential Scanning Calorimetry

**3) PREPARATION OF LORNOXICAM LOADED NIOSOMES**

Preparation of niosomal formulations using various ratios of non ionic surfactants and cholesterol by thin film hydration method.

**4) EVALUATION OF NIOSOMAL FORMULATION**

Drug Content Analysis

Estimation of Entrapment Efficiency

Invitro Drug Release Studies

Kinetics of drug release

**5) PREPARATION OF NIOSOMAL GEL FORMULATIONS**

Formulation of the best niosomal formulation in to gel form for topical drug delivery.

**6) EVALUATION OF NIOSOMAL GEL FORMULATIONS**

Drug content analysis

pH measurements

Rheological studies

Transmission electron microscopy

Particle size analysis

In-vitro release studies

Kinetics of drug release

Anti-inflammatory studies

# CHAPTER - VIII

# MATERIALS AND EQUIPMENTS

**MATERIALS USED:**

| | |
|---|---|
| Drug- lornoxicam | - Micro lab**,** Hosur India |
| Poly acrylic acid (Carbopol 940) | - Dr. Reddys Lab, Hyderabad |
| Cholesterol | - Sis co Research Lab, Mumbai |
| Sorbitonmonopalmitate | - S.D.fineChem, Mumbai |
| Sorbitonmonostearate | - S.D.fineChem, Mumbai |
| Sorbitonmonooleate | - S.D.fineChem, Mumbai |
| Tween-60 | - HimediaLab, Mumbai |
| Tween-40 | - Himedia Lab, Mumbai |
| Tween- 20 | - Reachem, Chennai |
| Tween- 80 | - Himedia Lab, Mumbai |
| Chloroform | - HPLC, Mumbai |
| Methanol | - Rankem, New Delhi |
| n-propanol | - Nice Chemical, Kochi |
| Sodium chloride | - Central Drug House, New Delhi |
| Potassium dihydrogen ortho phosphate | - HPLC, Mumbai |
| Disodium hydrogen ortho phosphate | - HPLC, Mumbai |
| Dialysis memebrane 50 – LA 387 | - Himedia Lab, Mumbai |
| Brij 52 | - Sigma Aldrichco, USA |
| Triethanolamine | - S.D.fineChem, Mumbai |

**EQUIPMENTS USED:**

| | |
|---|---|
| Rotary Flash Evaporator | - Super fit rotary flash evaporator, Mumbai |
| Ultra Sonicator | - Vibronic's Ultrasonic processor |
| Electronic Balance | - A&D Company, Japan |
| Magnetic Stirrer | - MC Dalal& co, Chennai |
| UV Visible Spectrophotometer | - UV Pharma Spec 1700, Shimadzu, Japan |
| Cooling Centrifuge Apparatus | - Eppendorf Centrifuge 5417R, Germany |
| Particle size analyzer | - Marlven,U.K |
| Transmission electron microscopy | - Hitachi S-3400, Japan. |
| FT-IR Spectrophotometer | - Shimadzu, Japan. |
| Differential Scanning Calorimeter | - Perkin ElmerSTA 6000, Mumbai. |
| Refrigerator | - Kelvinator, India. |
| Environmental chamber | - Inlabequipments (Madras) Pvt. Ltd, |
| $P^H$ Meter | - Dalal Company, Chennai. |

# CHAPTER-IX

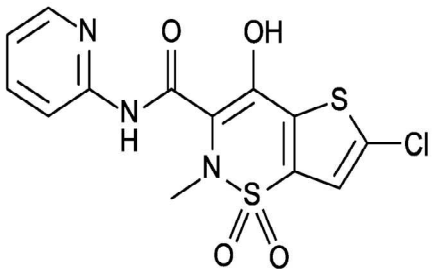
# DRUG PROFILE

**LORNOXICAM** [Haberfed, H, 2009**]**

**SYNONYM**

- Chlortenoxicam
- Lorcam
- Xefocam

**STRUCTURE**




**IUPAC NAME**

(3E)-6-Chloro-3-[hydroxyl (pyridine-2-ylamino) methylene]-2-methyl-2, 3-dihydro-4H-thieno [2, 3-e][1,2]thiazin-4-one 1,1-dioxide.

**CHEMICAL FORMULA**

$C_{13}H_{10}ClN_3O_4S_2$

**DESCRIPTION**

| | | |
|---|---|---|
| Nature | : | Yellow crystalline powder |
| Solubility | : | Soluble in 0.1N/NAOH, Soluble in chloroform, Insoluble in water |
| Melting point | : | 225-230°C |
| Molecular weight | : | 371.81gm/mol |
| Log P (octanol/$p^H$ 7.4 buffer) | : | 1.8 (Lornoxicam) |

PKa : 13.63 (Lornoxicam)

**CATEGORY**

- Non-steroidal anti-inflammatory drug.
- Antipyretic agent.

**IDENTIFICATION**

UV light absorption at 375 nm.

**PHARMACODYNAMIC PROPERTIES**

Lornoxicam NSAID that is used in musculoskeletal, joint disorders and other painful conditions including postoperative pain. Lornoxicam is a potent inhibitor both COX-1 and COX-2 enzyme.

**PHARMACOKINETIC PROPERTIES**

**Absorption**

- Lornoxicam is absorbed rapidly and almost completely from the gastro-intestinal tract.
- Maximum plasma concentrations are achieved after approximately 1 to 2 hours.

**Metabolism**

Lornoxicam is found in the plasma in unchanged from and as its hydroxylated metabolite. The hydroxylated metabolite exhibits no pharmacological activity. CYP2C9 has been shown to be the primary enzyme responsible for the biotransformation of the lornoxicam its major metabolite, 5-hydroxylornoxicam. Lornoxicam 5-hydroxylation by the variant CYP2C9*3 and CYP2C9*13 is markedly reduced compared with wild type, both in vivo and in vitro.

**Excretion**

Excreted in the feces (as metabolites) and urine (as unchanged drug). Mean elimination half life of 3-4 hours.

**Pharmacokinetic Characters of Lornoxicam**

| | | |
|---|---|---|
| Bioavailability | : | 90-100% |
| Protein binding | : | 99% |
| Metabolism | : | CYP2C9 |
| Half life | : | 3-4 hours |
| Excretion | : | 1/3 Renal, 2/3 Hepatic |

**THERAPEUTIC INDICATIONS**

- Lornoxicam is used for the treatment of various types of pain, especially resulting from inflammatory diseases of the joints, osteoarthritis, and other inflammations.
- Antipyretic agents.

**DOSE**

**Oral**

Pain relief 8-16 mg daily.maximum:24 mg daily (Adult). Osteoarthritis 12 mg daily in 2-3 divided doses, up to 16 mg daily if needed.(Adult)

**Parenteral**

8 mg once or twice daily by IM/IV injection. Maximum:24 mg daily (Adult)

**STORAGE**

Protected from light.

**SIDE EFFECTS**

- Abdominal pain,
- Diarrhea,

- Dizziess
- Dyspepsia
- Nausea
- Vomiting
- Headache
- Hematologic disorders
- CNS effects
- Tinnitus
- Visual disturbance
- Fluid retention
- Stomatitis
- Hypertension

**DRUG INTERACTIONS**

- Increased lornoxicam blood concentration when given concomitantly with cimetidine.
- Enhanced effects of anticoagulants, sulfonylurea, methotrexate, cyclosporine, and digoxin.
- Decreased effects of diuretics, ACE inhibitors

**SPECIAL PRECAUTIONS**

- Active infections
- Asthma
- Allergic disorders
- hemorrhagic disorders
- hypertension
- impaired renal

- hepatic
- cardiac function

**CONTRA INDICATIONS**

- Contraindicated in pregnancy,
- Contraindicated in lactation,
- Patients with peptic ulceration,
- Severe renal impairment.

**BRAND NAMES**

- Lorcam (Taisho Pharmaceutical Co.)
- Xafon (Nycomed)

# CHAPTER-X

# EXCIPIENTS PROFILE

**CHOLESTEROL** (Raymond C Rowe et al., 2006)

**SYNONYM**

- Cholesterin, Cholesterolum

**CHEMICAL NAME**

- Cholest -5- en-3β -ol.

**EMPIRICAL FORMULA**

- $C_{17}H_{46}O$

**MOLECULAR WEIGHT**

- 386.67

**FUNCTIONAL CATEGORY**

- Emollient
- Emulsifying agent

**DESCRIPTION**

- Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan color.

**PROPERTIES**

| | |
|---|---|
| Boiling Point | 360 $^{o}$C |
| Density | 1.052g/cm$^3$ for anhydrous form |
| Melting Point | 147-150$^{o}$C |
| Solubility | Soluble in acetone and vegetable oils. |

Practically insoluble in water and chloroform

**STABILITY AND STORAGE CONDITIONS**

- It is stable, and should be stored in a well-closed container and protected from light.

**SAFETY**

- It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

**HANDLING PRECAUTIONS**

- Rubber or plastic gloves, eye protection and a respirator are recommended.

**METHOD OF MANUFACTURE**

- The commercial material is normally obtained from the spinal cord of cattle by extraction with petroleum ether, but it may also be obtained from wool fat. Purification is normally accomplished by repeated bromination. Cholesterol may also be produced by entirely synthetic means.

**REGULATORY STATUS**

- Induced in the FDA inactive ingredients.

## POLYSORBATE 20

### SYNONYM

- Armotan PML 20, Capmul POE-l, TW 20, T-MAZ-20, Tween 20.

### CHEMICAL NAME

- Polyoxy ethylene 20 sorbitan mono laurate sorbitan mono decanoate

### EMPIRICAL FORMULA

- $C_{58}H_{114}O_{26}$

### MOLECULAR WEIGHT

- 1128

### DESCRIPTION

- It is Yellow oily liquid and having characteristic odour and bitte

### METHOD OF MANUFACTURE

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterified with a fattyacid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates.

### PROPERTIES

| | |
|---|---|
| Acid value | 2.0 |
| Hydroxyl value | 96 – 108 |
| Density ($g/cm^3$) | $1.1 g/cm^3$ |
| HLB Value | 16.7 |
| Solubility | Soluble in ethanol and water. Insoluble in mineral oil and vegetable oil. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Useful to improving oral bioavailability of drug molecule
- Widely used in cosmetics and food products.

**STABILITY**

- Stable to electrolytes and weak acids and bases, Gradual saponification occur with strong acids or bases.
- Hygroscopic
- Prolonged storage leads to the formation of peroxides.

**STORAGE**

- It should be stored in a well-closed container and protected from light

**SAFETY**

- Widely used in cosmetics, food products, oral, parenteral and topical pharmaceutical formulations and generally regarded as non-toxic and non-irritant materials.
- Daily intake according to the WHO limit is 25mg/Kg body weight.
- $LD_{50}$ (rat, oral) is about 37gm/Kg.

H**ANDLING PRECAUTIONS**

- Eye protection and Gloves are recommended (Raymond C Rowe et al.,2006)

## POLYSORBATE 40

**SYNONYM**

Crillet 2, E434, sorbox PMP, Tween 40.

**CHEMICAL NAME**

Sorbitan monohexa decanoate.

**EMPIRICAL FORMULA**

$C_{62}H_{122}O_{26}$

**MOLECULAR WEIGHT**

1284

**DESCRIPTION**

Yellow oily liquid.

**METHOD OF MANUFACTURE**

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterified with a fattyacid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the Polysorbates

**PROPERTIES**

| | |
|---|---|
| Acid value | 2.0 |
| Hydroxyl value | 90 – 105 |
| Saponification value | 41 - 52 |
| Density ($g/cm^3$) | $1.08 g/cm^3$ |
| HLB Value | 15.6 |

| | |
|---|---|
| Solubility | Soluble in ethanol and water. Insoluble in mineral oil and Vegetable oil. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent
- Nonionic Surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

**STABILITY**

- Gradual soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

**STORAGE**

- It should be stored in a well-closed container in a cool, dry place.

**SAFETY**

- Daily intake according to the WHO limit is about 25mg/Kg body weight and moderately toxic by IV route.

**HANDLING PRECAUTIONS**

- Eye protection and Gloves are recommended.

## POLYSORBATE 60

### SYNONYM

Atlas 70k, Atlas Armotan PMS 20, Glycosporse s-20, Tween 60, Tween 60k, Tween 60VS.

### CHEMICAL NAME

Sorbitanmono Octadecanoate.

### EMPIRICAL FORMULA

$C_{64}$ $H_{126}$ $O_{26}$

### MOLECULAR WEIGHT

1312

### DESCRIPTION

Yellow oily liquid.

### METHOD OF MANUFACTURE

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterified with a fattyacid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates.

### PROPERTIES

| | |
|---|---|
| Acid value | 2.0 |
| Hydroxyl value | 81 – 96 |
| Saponification value | 45 - 55 |
| Density ($g/cm^3$) | $1.1 g/cm^3$ |
| HLB Value | 14.9 |

| | |
|---|---|
| Solubility | Soluble in ethanol and water. Insoluble in mineral oil and Vegetable oil. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

**STABILITY**

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

**STORAGE**

- It should be stored in a well-closed container in a cool, dry place.

**SAFETY**

- Daily intake according to the WHO limit is about 25mg/Kg body weight and moderately toxic by IV route.

**HANDLING PRECAUTIONS**

- Eye protection and Gloves are recommended.

## POLYSORBATE 80

### SYNONYM

Atlas E, Capmul POE-o, Glycosporse o-20, Tego SMO 80, Tego SMO 80 x, Tween 80.

### CHEMICAL NAME

(Z) Sorbitan mono-9- Octadecanoate poly (oxy 1, 2, ethanediyl) derivatives.

### EMPIRICAL FORMULA

$C_{64}$ $H_{124}$ $O_{26}$

### MOLECULAR WEIGHT

1310

### DESCRIPTION

Yellow oily liquid.

### METHOD OF MANUFACTURE

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterifies with a fatty acid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates

### PROPERTIES

| | |
|---|---|
| Acid value | 2.0 |
| Hydroxyl value | 65 – 80 |
| Saponification value | 45 - 55 |
| Density ($g/cm^3$) | $1.08 g/cm^3$ |
| HLB Value | 15 |

| | |
|---|---|
| Solubility | Soluble in ethanol and water. Insoluble in mineral oil and Vegetable oil. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

**STABILITY**

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

**STORAGE**

- It should be stored in a well-closed container in a cool, dry place.

**SAFETY**

- Daily intake according to the WHO limit is about 25mg/Kg body weight
- $LD_{50}$ (Mouse, oral)-25g/Kg.

**HANDLING PRECAUTIONS**

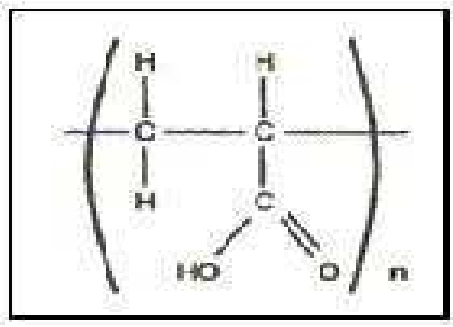
- Eye protection and Gloves are recommended.

**REGULATORY STATUS**

Polysorbates 20,40,60,65 and 80 are accepted as food additives in Europe. Polysorbates 20, 40, 60, and 80 are included in the FDA inactive ingredients guide (IM, IV, Oral, rectal, topical and vaginal preparations). Polysorbates are included in parenteral and non-parenteral medicines licensed in the UK.

## CARBOPOL 940

### STRUCTURE




### SYNONYMS

Acritamer; acrylic acid polymer; Carbopol; carboxypolymethylene,polyacrylic acid; carboxyvinyl polymer; Pemulen;Ultrez. Chemical Name and CAS Registry Number

Carbomer

### EMPIRICAL FORMULA AND MOLECULAR WEIGHT

Carbomer are synthetic high-molecular-weight polymers of acrylic acid that are cross linked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid (COOH) groups 104 400 g/mol for Carbopol 940 have been reported

### STRUCTURAL FORMULA

Carbomer polymers are formed from repeating units of acrylic acid. The polymer chains are cross linked with allyl sucrose or allyl pentaerythritol.

### FUNCTIONAL CATEGORY

Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent.

## APPLICATIONS IN PHARMACEUTICAL FORMULATION OR TECHNOLOGY

Carbomer are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams, gels, and ointments for use in ophthalmic, (5–7) rectal, (8–10) and topical preparations.

## EMULSIFYING AGENT

0.1–0.5

## GELLING AGENT

0.5–2.0

## SUSPENDING AGENT

0.5–1.0

## TABLET BINDER

5.0–10.0

## DESCRIPTION

Carbomer are white-colored, ‘fluffy’, acidic, hygroscopic powders with a slight characteristic odor.

## PHARMACOPEIAL SPECIFICATIONS

Carbomer 940 (0.5 w/v) — 40 000–60 000(a)

## TYPICAL PROPERTIES

### Acidity/alkalinity

pH = 2.7–3.5 for a 0.5% w/v aqueous dispersion;

PH = 2.5–3.0 for a 1% w/v aqueous dispersion.

### Density (bulk)

1.76–2.08 g/cm3

**Density (tapped)**

1.4 g/cm3

**Glass transition temperature**

100–105ºC

**Melting point**

Decomposition occurs within 30 minutes at 260ºC.

**Moisture content**

Normal water content is up to 2% w/w.However, Carbomer are hygroscopic and typical equilibrium moisture content at 25ºC and 50% relative humidity is 8–10% w/w. The moisture content of a Carbomer does not affect its thickening efficiency (Raymond C Rowe et al., 2006).

## SORBITAN MONOPALMITATE

### SYNONYMS

Ablunol S-40, Armotan MP, Liposorb P, Span 40, Arlacel 40, Montane 40, Sorbitan Palmitate.

### CHEMICAL NAME

Sorbitan monohexa decanoate.

### EMPIRICAL FORMULA

$C_{22}H_{42}$ O6

### MOLECULAR WEIGHT

403

### DESCRIPTION

It occurs as cream solid with a distinctive odour and taste.

### METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexiton (1,4 Sorbitan) which is then esterified with the desired fatty acid.

### PROPERTIES

| | |
|---|---|
| Acid value | 3 to 7 |
| Hydroxyl value | 270 to 303 |
| Iodine value | ≤ 1 |
| Density ($g/cm^3$) | 1.0 |
| HLB Value | 6.7 |
| Melting point | $43^0C$ - $48^oC$ |
| Solubility | Soluble in oils and in organic solvents. Insoluble in water. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent.
- Non ionic Surfactant.
- Solubilizing agent.
- Wetting agent.

**STABILITY**

It should be stored in a well-closed container in a cool, dry place.

**SAFETY**

It is generally regarded as non-toxic and non-irritant material.

**HANDLING PRECAUTIONS**

Eye protection and Gloves are recommended (Raymond C Rowe et al., 2006).

## SORBITAN MONOSTEARATE

### SYNONYMS

Ablunol S-60, Alkamuls SMS, Sorgen 50, Tego SMS, Span 60, Arlacel 60, Durtan 60, Montane 60, Sorbitan Stearate.

### CHEMICAL NAME

Sorbitan mono – Octadecanoate.

### EMPIRICAL FORMULA

$C_{24}H_{46}O_6$

### MOLECULAR WEIGHT

431

### DESCRIPTION

It occurs as a cream solid with a distinctive odour and taste.

### METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexiton (1,4 Sorbitan) which is then esterified with the desired fatty acid.

### PROPERTIES

| | |
|---|---|
| Acid value | 5 to 10 |
| Hydroxyl value | 235 to 260 |
| Iodine value | ≤ 1 |
| HLB Value | 4.7 |
| Melting Point | $53^0C – 57^oC$ |
| Solubility | Soluble in oils and in most organic solvents. Insoluble but dispersible In water. |

**FUNCTIONAL CATEGORY**

- Emulsifying agent.
- Nonionic Surfactant.
- Solubilizing agent.
- Wetting agent.

**STABILITY**

- Gradual Soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

**STORAGE**

It should be stored in a well-closed container in a cool, dry place.

**SAFETY**

- It is generally regarded as non-toxic and non-irritant material.
- Very mildly toxic by ingestion.

**HANDLING PRECAUTIONS**

Eye protection and Gloves are recommended.

## SOBITAN MONO OLEATE

### SYNONYMS

Ablunol S-80, Armotan MO, Capmul O, Crill 4, Lames orb SMO, Span 80, Arlacel 80, Montane 80, Sorgen 40, Sorbitan Oleate.

### CHEMICAL NAME

(Z) - Sorbitan mono -9- Octa deaconate.

### EMPIRICAL FORMULA

$C_{24}$ $H_{44}$ $O_6$

### MOLECULAR WEIGHT

429

### DESCRIPTION

It occurs as yellow viscous liquid with a distinctive odour and taste.

### METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexiton (1,4 Sorbitan) which is then esterified with the desired fatty acid.

### PROPERTIES

| | |
|---|---|
| Acid value | ≤ 8 |
| Hydroxyl value | 193 to 209 |
| Pour point | 12 |
| Density ($g/cm^3$) | 1.01 |
| HLB Value | 4.3 |
| Solubility | Soluble in oil and in most organic solvents.<br>Insoluble but dispersible in water. |

**FUNCTIONAL CATEGORY:**

- Emulsifying agent.
- Nonionic surfactant.
- Solubilizing agent.
- Wetting agent.

**STABILITY:**

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

**STORAGE:**

It should be stored in a well-closed container in a cool, dry place.

**SAFETY:**

It is generally regarded as non-toxic and non-irritant material.

**HANDLING PRECAUTIONS:**

Eye protection and Gloves are recommended (Raymond C Rowe et al., 2006).

# CHAPTER-XI

# EXPERIMENTAL PROTOCOL

## 1) STANDARD CURVE FOR LORNOXICAM

**Preparation of calibration medium**

***Phosphate buffered saline pH 7.4***

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient quantity of distilled water and the volume made up to1000 ml.

**Determination of absorption maximum ($\lambda_{max}$) by UV spectrum**

UVspectrum is obtained for 10µg/ml concentration of lornoxicam using standard buffer solution (Phosphate buffered saline pH 7.4).

**Calibration curve for Lornoxicam**

100mg of Lornoxicam is accurately weighed and dissolved in a small quantity of methanol and made up to 100ml with the phosphate buffered saline pH 7.4. From this primary solution 10ml is pipetted out and made up to 100ml with phosphate buffered saline pH 7.4. From this secondary solution aliquots are taken to produce 2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg/ml.

The absorbance of the resulting solution is measured at 375nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using phosphate buffered saline pH 7.4 as blank ( Metker Vishal et al.,2011). The standard curve is plotted by taking concentration in X-axis and Absorbance in Y-axis.

## 2) DRUG –EXCIPIENTS INTERACTION STUDIES

***Fourier Transform-Infrared Spectroscopic Studies (FT-IR)***

The possibility of drug-excipients (cholesterol, nonionic surfactants) interactions are further investigated by FT-IR study. The FT-IR spectrum of pure drug and combination of drug with excipient are obtained by using JASCO FT-IR Spectrophotometer. The scanning range is 400-4000 $cm^{-1}$ and the resolution is $1cm^{-1}$(Md.Ismail Mouzan *et al*., 2010). Samples is prepared in KBr pellets (Jasco FT-IR 5300 Japan).

***Differential Scanning Calorimetry***

Thermogram of the pure drug, Cholesterol, non ionic surfactant and physical mixtures (drug, surfactants and cholesterol) are obtained using a shimadzu W70 thermal analyzer for differential scanning calorimeter. Samples (4-8mg) are sealed into aluminum pans and measurements are run from 25º to 300º C against an empty pan (Md.Ismail Mouzan *et al*., 2010).

## 3) PREPARATION OF LORNOXICAM LOADED NIOSOMES:

***Thin Film Hydration Method***

Different ratios of surfactant and cholesterol are used to prepare niosomes with the concentration of the drug being the same.

The niosome formulations are prepared by thin film hydration technique. The weighed amount of cholesterol, non-ionic surfactant dissolved in 5ml of solvent mixture (Chloroform: Methanol 2:1 ratio). 20 mg of lornoxicam is dissolved in 20ml of chloroform to make clear solution. It is then transferred into a 100ml round bottom flask. A thin film is formed under reduced pressure in a rotary flash evaporator rotated at 100rpm at 55ºC. The organic solvent is evaporated to form a dry film on the walls of the flask. An appropriate amount of phosphate buffered saline pH 7.4 is added slowly to the round bottom flask having thin film of surfactant and cholesterol and vortexed continuously for a period of 45 minutes at

55ºC, until a good dispersion of the mixture is obtained. The niosomal dispersion is collected and stored at 4ºC for maturation (Vijay Prakash Pandey *et al*., 2009, Vijay S .Jatav et al., 2011, Vyas Jigar *et al.*, 2011). The empty niosomes also prepared by the same method without the drug for further evaluation.

**4) EVALUATION OF NIOSOMAL FORMULATION:**

***Drug Content Analysis***

The amount of drug in the formulation is determined after lysing the niosomes using 50% n- propanol. Niosomes preparation equivalent to 200 µg of Lornoxicam (1ml) is pipetted out in 100 ml standard flask. To this sufficient quantity of 50% n- propanol is added and shaken well for the complete lyses of the vesicles. The volume is made up to 100 ml with the buffer phosphate buffered saline pH 7.4. The absorbance is measured at 375nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using empty niosomes as blank. The drug content is calculated from the standard curve, by using the following formula,

$$\textbf{Drug content} = \frac{\textbf{Sample Absorbance}}{\textbf{Standard Absorbance}} \textbf{X 100}$$

***Estimation of Entrapment Efficiency***

Lornoxicam niosome preparations (1 ml) are centrifuged at 14,000 rpm for 120 minutes at 4ºC using a refrigerated centrifuge (Eppendorf, 5417R, Germany) in order to separate niosomes from unentrapped drug (Kandasamy Ruckmani *et al*., 2010, Meenakshi Chauhan *et al*., 2009). The free drug concentration in supernatant layer after centrifugation is determined at 375 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). The percentage of drug entrapment in niosomes is calculated using the following formula,

$$\% \text{ drug entrapment} = \frac{\text{(Total drug – Unentrapped drug)}}{\text{Total drug}} \times 100$$

***Invitro Drug Release Studies***

Invitro release pattern of niosomes suspension is carried out by dialysis bag (Himedia M.W 12000). The niosomal preparation of Lornoxicam is placed in a dialysis bag with an effective length of 5 cm which acts as a donor compartment. Dialysis bag is placed in a beaker containing 250 ml of buffer phosphate buffered saline pH 7.4, which acts as receptor compartment. The temperature of receptor medium maintained at 37±1ºC and the medium is agitated at 50 rpm speed using magnetic stirrer. Aliquots of 5 ml samples are collected at predetermined time and replenished immediately with the same volume of fresh phosphate buffered saline pH 7.4. The sink condition is maintained throughout the experiment. The collected samples are analyzed spectrophotometrically at 375 nm using UV-Visible Spectrophotometer (ShimadzuUV-1700 Pharma spec Japan). Each study is performed in triplicate (Manivannan Rangasamy *et al*., 2008, Yasmin Begum *et al*., 2011). The invitro release studies are also carried out for the pure drug by same method.

***Kinetics of drug release***

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes are fit with various pharmacokinetic equations like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), and the korsmeyer-peppas (log cumulative % drug release vs. log time) and Hixson- Crowell models (cubic root of drug remaining Vs time). The $r^2$ and k values were calculated for the linear curve obtained by

regression analysis (Meenakshi Chauhan *et al*., 2009, Rathi Jagdish Chandra *et al*., 2009, Ibrahim A. Alsarra *et al*., 2005 Tamizharasi *et al*., 2009).

## 5) PREPARATION OF NIOSOMAL GEL FORMULATIONS

***Preparation of Lornoxicam niosomal gel***

Carbopol 940 gel bases are prepared by homogenizing 1 % (w/w) carbopol dispersion in sufficient water using a magnetic stirrer for 30 minutes and leaving it to equilibrate for 24 hours. After that, pH is adjusted to 5 – 7 with Triethanolamine (Saleem M.A, *et al*., 2010). The lornoxicam loaded niosomes is added to the prepared plain gel base during the stirring process and the step is completed as mentioned for carbopol plain gel bases.

## 6) EVALUATION OF NIOSOMAL GEL FORMULATIONS

***Drug content analysis***

An accurately weighed quantity of each Lornoxicam niosomal gel (100 mg) is dissolved in 50 ml of phosphate buffer (pH 7.4). These solutions are quantitatively transferred to volumetric flask and appropriate dilutions are made with the same buffer solution. (Fathy I. Abd-Allah *et al*., 2010). The resulting solutions are then filtered through membrane filters (pore size 0.45 mm) before subjecting the solution to spectro photometric analysis for Lornoxicam at 375 nm (Shimadzu UV-VIS spectrophotometer).

***pH measurements***

The pH is measured in each niosomal gel using a pH meter. This is calibrated before each use with buffered solutions at pH 4, 7 & 10. 1 gram of gel is taken and diluted with 100 ml of distilled water and stored for two hours. The electrode of the pH meter is immersed in the prepared base solution for pH determination. The pH determination is carried out in triplicate and the average reading is recorded (Prabhudutti Panda *et al*., 2010).

***Rheological studies***

The viscosity of gel formulation is carried out on Brook-field viscometer using spindle number S-06, and the determinations is carried out in triplicate and the average of three reading is recorded (Fathy I. Abd- Allah *et al*.,2010).

***Transmission electron microscopy***

The morphology of the lornoxicam niosomal gel dispersions are determined by transmission electron microscopy. A drop of niosomal gel dispersion is applied to a carbon coated 300 – mesh copper grid and left to adhere on the carbon substrate for about 1 min.The remaining gel dispersion was removed by a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 35 seconds and again the solution in excess was removed by the tip of filter paper (RitaMuzzalupo *et al*., 2008, Muzzalupo *et al*., 2011). The sample was air dried and observed under the transmission electron microscope at 90 kV.

***Particle size analysis***

The vesicle size distribution is determined using a laser technique on a master sizer. (X Ver.2.15; Malvern instrunents Ltd. Malvern, UK). The measurements were performed at 25º C using a 45 mm focus lens and a beam length 2.4 mm (Mohamed Nasr *et al*., 2009).

***In-vitro release studies***

*In-vitro* release study is carried out by taking 1 g of gel formulations into dialysis bag and placed beaker containing 100 ml PBS pH 7.4 at 37 ± 10˚C. The beakers placed over a magnetic stirrer and stirred at 50 rpm. Aliquots of samples are withdrawn at specified time intervals and analyzed at 375 nm by using an UV spectrophotometer to determine the percentage drug released and replaced with equal volume of fresh PBS pH7.4 (A. Abdul Hasan Sathali *et al*., 2010).

***Kinetics of drug release***

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes gel are fit with various pharmacokinetic equations like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), and the korsmeyer-peppas (log cumulative % drug release vs. log time) and Hixson- Crowell models (cubic root of drug remaining Vs time). The $r^2$ and k values were calculated for the linear curve obtained by regression analysis (Meenakshi Chauhan et al., 2009, Rathi Jagdish Chandra *et al*., 2009, Ibrahim A. Alsarra *et al*., 2005 Tamizharasi *et al*., 2009).

***Anti-inflammatory studies***

The studies were conducted on albino rats of either sex, weighing 150-200g. The animals in each were selected so that the average body weight among the groups as close as possible. (Ref No 14024/E1/4/2011) . Inflammation is produced in the rats by injecting 0.1ml of 1% w/v carrageenam niosomal gel formulations and plain gel (Drug dispersion in carbopol) and without drug gel was applied topically on the edematous paw by gently rubbing with an index finger. Topical activities of the various formulations were evaluated by measuring an increase in the hind paw thickness with the help of digital plethesmograph. Before and after (1, 2, 3, 4, 6, and 24 hours) carrageenam administration. The percentage of paw thickness increase from time 0 were calculated with compared with control group.

***Group-1(Control)***

Animal were treated with carrageenam

***Group-2 (Standard)***

Animal were treated with Lornoxicam plain gel.

***Group-3 (Test-1)***

Animals were treated with Test-1(High entrapment niosomal gel).

***Group-4 (Test-2)***

Animal were treated with Test-2(Low entrapment niosomal gel)

The percentage inhibitions of paw thickness were calculated by the following formula: (Munish Ahuja *et al*., 2011).

**Percentage inhibition = [C –T / C] × 100**

Where;

C = Control Paw edema

T = Test Paw edema

# CHAPTER-XII

# RESULTS AND DISCUSSION

## 1) STANDARD CURVE FOR LORNOXICAM

### Preparation of calibration medium

The preparation of calibration medium was prepared by using phosphate buffer saline pH 7.4.

### Determination of absorption maximum ($\lambda_{max}$) by UV spectrum

The $\lambda_{max}$ of Lornoxicam was determined by scanning the 10µg/ml of drug solution in phosphate buffered saline (PBS) pH 7.4 at 375 nm in UV visible spectrophotometer (Metker Vishal *et al*., 2010). The graph was shown in Figure 7

### Calibration curve for Lornoxicam

Calibration curve of Lornoxicam was plotted by measuring the absorbance of different concentrations of the drug in phosphate buffered saline pH at 375 nm in the UV- visible spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). The Lornoxicam obeys the Beers law within the concentration of 2 to 20 µg/ml. The $\lambda_{max}$ at UV region of 375 nm. The calibration data and graph were shown in Table 3 and Figure 8

## 2) DRUG –EXCIPIENTS INTERACTION STUDIES

### *Fourier transform- Infra red spectroscopic studies (FT-IR)*

FT-IR spectrum of pure drug, surfactants, cholesterol and physical mixtures (drug, surfactant and cholesterol). From this study it was observed that there was no significant

interaction between the pure drug and the physical mixture used in the formulations. (Rathi Jagdish Chandra *et al*., 2009).The results were shown in Figure 9A- I

***Table No: 2 The FT-IR spectra of lornoxicam drug show as follows:***

| ***S. No*** | ***Wave Number ($cm^{-1}$).*** | ***Bond*** |
|---|---|---|
| *1.* | 3090 | NH stretching |
| *2.* | 1642 | C=O group in the primary amide |
| *3.* | 1597, 1559 | Bending vibrations of the N–H group in the secondary amide |
| *4.* | 1157, 1387 and 1336 | Stretching vibrations of the O=S=O |
| *5.* | 827.94 | –CH aromatic ring bending and heteroaromatics |
| *6.* | 766.8 | C–Cl bending vibration |

***Differential scanning Calorimetry***

DSC studies are useful method of detecting drug-excipient incompatibility. DSC Thermogram of the pure drug (Lornoxicam), Non-ionic surfactants (Span 40, Span 60, Tween 20, and Tween 40), cholesterol and physical mixtures are shown in the Figure: 10A- I. Drug showed the sharp melting endothermic peak at 221.61º C. The non-ionic surfactants Span 40, Span 60, Tween 20 and Tween 40 showed the sharp melting endothermic peak at 48º C, 57º C,

134ºC and 51.45ºC. The physical mixtures Span60, cholesterol and lornoxicam showed the sharp melting endothermic peak at 59.19ºC, the physical mixtures Tween 60, cholesterol and lornoxicam showed the sharp melting endothermic peak at 69.79ºC and the physical mixtures Tween 80, cholesterol and lornoxicam showed the sharp melting endothermic peak at 83.40ºC. It suggests that the formulation components Span 60, Span 40, Tween 20, Tween 40, cholesterol and the drug lornoxicam do not interact to form any additional chemical entity but remain as mixture. (Md.Ismail Mouzan et al., 2010).

**3) PREPARATION OF LORNOXICAM LOADED NIOSOMES**

In the present study, thirty two niosomal formulations were prepared by thin film hydration method using different ratios of non-ionic surfactants (Span 40,60 and 80, Tween 20, 40, 60, 80 and Brij-52 ) along with Cholesterol(25µmol) and concentration of the drug(2mg/ml) being constant .The formula were shown in Table 4,5 and 6.

**4) EVALUATION OF NIOSOMAL FORMULATION:**

***Drug content analysis:***

The percentage drug content of all the formulations (F1 – F32) were found to be range 95% to 96.95% ensured the uniformity of drug content in all formulations. The results were shown in Table 18

***Estimation of Entrapment efficiency***

The entrapment efficiency of the prepared niosomal formulations were measured by centrifugation method. The entrapment efficiency was determined by subtracting the amount of drug entrapped from the total amount of drug in the formulation. The results of the niosomal

formulations maximum entrapment obtained for the formulation containing (F32) Brij-52 and cholesterol in the ratio (10:1) was 92.05%. This may be attributed to its length of longer side chain (lauryl), and it easily diffuse into receptor membrane integrity, orientation and packaging ability of Brij- 52 ( Kumar *et al*., 2012). In all the formulation, the impact of surfactants on entrapment efficiency was significant.

**Effect of non ionic surfactant on entrapment efficiency**

The entrapment efficiency of niosomal formulations were (Span 40: Cholesterol) 75.86%, 76.38%, 77.13% and79.26% for formulationsF1 (4:1), F2 (6:1), F3 (8:1) and F4 (10:1) respectively and the orders as follows.

**F1 < F2 < F3 < F4**

The entrapment efficiency of niosomal formulations were (Span 60: Cholesterol) 84.38%, 86.69%, 87.06% and 89.91% for formulations F4 (4:1), F6 (6:1), F7 (8:1) and F8 (10:1) respectively and the orders as follows.

**F5 < F6 < F7 < F8**

The entrapment efficiency of niosomal formulations were (Span 80: Cholesterol) 66.75% 68.48%, 69.51% and 70.27% for formulations F9 (4:1), F10 (6:1), F11 (8:1) and F12 (10:1) respectively and the orders as follows.

**F9 < F10 < F11 < F12**

The entrapment efficiency of niosomal formulations were (Tween 20: Cholesterol) 70.13%, 71.14%, 72.31% and 73.37% for formulations F13 (4:1), F14 (6:1), F15 (8:1) and F16 (10:1) respectively and the orders as follows.

**F13 < F14 < F15 < F16**

The entrapment efficiency of niosomal formulations were (Tween 40: Cholesterol) 74.03%, 75.37%, 77.28% and 79.23% for formulations F17 (4:1), F18 (6:1), F19 (8:1) and F20 (10:1) respectively and the orders as follows.

**F17 < F18 < F19 < F20**

The entrapment efficiency of niosomal formulations were (Tween 60: Cholesterol) 86.69%, 87.44%, 88.72% and 89.48% for formulations F21 (4:1), F22 (6:1), F23 (8:1) and F24

(10:1) respectively and the orders as follows.

**F21 < F22 < F23 < F24**

The entrapment efficiency of niosomal formulations were (Tween 80: Cholesterol) 67.65%, 69.51%, 70.55% and 71.24% for formulations F25 (4:1), F26 (6:1), F27 (8:1) and F28 (10:1) respectively and the orders as follows.

**F25 < F26 < F27 < F28**

The entrapment efficiency of niosomal formulations were (Brij 52: Cholesterol) 88.19%, 89.21%, 90.25% and 92.05% for formulations F29 (4:1), F30 (6:1), F31 (8:1) and F32 (10:1) respectively and the orders as follows.

**F29 < F30 < F31 < F32**

From the above results it was observed that increase in the concentration of non ionic surfactant, increased the entrapment efficiency of niosomes formulations. (A. Abdul Hasan Sathali *et al*., 2010).

These results explained that the brij-52 has higher entrapment efficiency than other span series and Tween series .This could be due to variation in the surfactant chemical structure. Increasing the alkyl chain length is leading to higher entrapment efficiency. The entrapment efficiency followed the trend **Brij-52 > Sp 60 > Tween 60 > Tween 40 > Sp 40 > Tween 20 > Tween 80 > Sp 80.** Among the all surfactants Span 80 showed low entrapment efficiency due to introduction of double bonds into the paraffin chains. Addition, niosome loaded with lornoxicam confirming the hypothesis that entrapment efficiency may be correlated with the hydrophobicity of the alkyl chain (Kumar *et al*., 2012). The results were shown in Table 7, 8 and 9. The result was shown in Figure 11.

***Invitro Drug Release studies***

The invitro release study of Lornoxicam loaded niosomes by dialysis bag method using phosphate buffered saline (PBS) pH7.4.

The Lornoxicam pure drug solution showed invitro release of 98.33% within 5 hours. The invitro release of Lornoxicam loaded niosomes was slower and controlled than the Lornoxicam pure drug solution (0.2mg/ml).

The cumulative percentage release of niosomal formulations were (Span 40: Cholesterol) 95.63%, 87.56%, 79.30% and 72.70% for formulations F1 (4:1), F2 (6:1) F3 (8:1) and F4 (10:1) at 12 hours respectively.

**F1> F2 > F3 > F4**

The cumulative percentage release of niosomal formulations were (Span 60: Cholesterol) 77.46%, 71.27%, 63.27% and 57.19% for formulations F5 (4:1), F6 (6:1), F7 (8:1) and F8 (10:1) at 12 hours respectively.

**F5 > F6 > F7 > F8**

The cumulative percentage release of niosomal formulations were (Span 80: Cholesterol) 94.4%, 90.9%, 85.7% and 74.30% for formulations F9 (4:1), F10 (6:1), F11 (8:1) and F12 (10:1) at 12 hours respectively.

**F9 > F10 > F11 > F12**

The cumulative percentage release of niosomal formulations were (Tween 20: Cholesterol) 97.8%, 86.9%, 80.0% and 61.5% for formulations F13 (4:1), F14 (6:1), F15 (8:1) and F16 (10:1) at 12 hours respectively.

**F13 > F14 > F15 > F16**

The cumulative percentage release of niosomal formulations were (Tween 40: Cholesterol) 98.0%, 84.8%, 82.15% and 67.4% for formulations F17 (4:1), F18 (6:1), F19 (8:1) and F20 (10:1) at 12 hours respectively.

**F17 > F18 > F19 > F20**

The cumulative percentage release of niosomal formulations were (Tween 60: Cholesterol) 90.20%, 86.10%, 81.40% and 76.0% for formulations F21 (4:1), F22 (6:1), F23 (8:1) and F24 (10:1) at 12 hours respectively,

**F21> F22 > F23 > F24**

The cumulative percentage release of niosomal formulations were (Tween 80: Cholesterol) 89.50%, 85.81%, 77.58% and 72.33% for formulations F25 (4:1), F26 (6:1), F27 (8:1) and F28 (10:1) at 12 hours respectively.

**F25 > F26 > F27 > F28**

The cumulative percentage release of niosomal formulations were (Brij 52: Cholesterol) 87.54%, 77.44%, 75.99% and 68.97% for formulations F29 (4:1), F30 (6:1), F31 (8:1) and F32 (10:1) at 12 hours respectively.

**F29 > F30 > F31 > F32**

From the above results, it was showed that increase in surfactant concentration, decreased the drug release. Important changes in release were observed upon changing the nature of the surfactant used in the Lornoxicam loaded niosomes. The experimental studies of niosomal formulations showed that the rate of drug release depends on the percentage of drug entrapment efficiency (Manivannan Rangasamy et al 2008). The results were shown in Table 10, 11, 12, 13,14,15,16 and 17. The result was shown in Figure 12A-H

***Effect of surfactant concentration in invitro drug release***

Increase the molar ratio of nonionic surfactant in niosomes controlled the drug release from niosomal vesicles. The presence of high molar ratio of non-ionic surfactant in F8(10:1), F16(10:1), F20(10:1), F32(10:1), F28(10:1), F4(10:1), F12(10:1) , F24(10:1) and exhibit the maximum drug release after 12 hrs about 57.19%, 61.5%, 67.47%, 68.97%, 72.33%,72.7% 74.30%, 76.0% and respectively. These similar results were shown in (Kandasamy Ruckmani *et al*., 2010).

**F8 < F16 < F20 < F32 < F28 < F4 < F12 < F24**

This is might be due to the high concentration of surfactant in the formulations. The hydrophilic surfactant having more HLB values may be the reason for more hydrophilic and high swelling of vesicles and higher release of drug after 12 hours than the other surfactant containing niosomes. The entire amount of loaded drug was not released from the niosomes. This is may be due to entrapment of the drug in the lipophilic region. The release also depends of alkyl chin length of the surfactant. (Kumar *et al*., 2012).The result was shown in Figure 13.

***Kinetics of drug release***

Linear regression analysis for the release was done to determine the proper order of drug release. All the formulations follow zero order kinetics. Calculation of Higuchi correlation coefficient confirms that the drug release was proportional to the square root of time indicating that lornoxicam release from niosomes was diffusion controlled. The formulation F1, F9, F10, F13, F17, and F21 was followed Higuchi model. The n value from the Korsmeyer- Peppas model for lornoxicam niosomal formulation were between 0.681 - 0.999 which indicated the formulations F2, F3, F4, F5, F6, F7, F11, F12, F14, F15, F18, F19, F20, F22, F23, F25, F28,F29, F30 and F31 followed the Non-Fickian mechanism. The formulations F8, F24, F26, F27 and F32 followed the super case II mechanism. (Kandasamy Ruckmani *et al*., 2010, Y.Anand Kumar *et al*., 2010). The results were shown in Table 19, 20, 21 and 22 Figure 14A-E.

## 5) PREPARATION OF NIOSOMAL GEL FORMULATIONS

***Preparation of Lornoxicam niosomal gel***

The selected niosomal formulation F8(Span 60:Cholesterol)(10:1) and F24(Tween 60:Cholesterol)(10:1) (on the basis of entrapment efficiency ,in vitro drug release) was incorporated in to suitable gel base (carbopol940) 1% to obtain 0.02% of the drug and plain Lornoxicam gel was prepared by incorporating the drug in to suitable gel base to obtain same 0.02% of the drug. The results were shown in Table 23.

## 6) EVALUATION OF NIOSOMAL GEL FORMULATIONS

***Drug Content analysis***

The percentage drug content of all the formulations (FG1, FG2 and FG3) were found to be 95.4%, 96.2% and 95% ensured the uniformity of drug content in all formulations. The results were shown in Table 24.

***pH measurements***

The formulationFG1, FG2 and FG3 were smooth and homogeneous appearance. They were easily spreadable with acceptable. The pH values ofFG1, FG2 and FG3 formulations ph 6.8. This is considered acceptable to avoid the risk of irritation upon application to the skin. The results were shown in Table 25.

***Rheological Studies***

A viscometer (Brookfield+ II LV viscometer) was used to measure the viscosities (in cps) of the gels. The spindle (TF 64) was rotated at 0.5 rpm upto 100 rpm. Samples of the gels were

to settle over 30 min at the assay temperature (28 ±/1ºC) before the measurements were taken. The viscosity of FG2 gel was found to be 20000 cps in0.5 rpm.The viscosity of FG3 gel was found to be 19500 cps in 0.5 rpm (Japan Patel et al., 2011). The results were shown in Table 26.

***Transmission electron microscopy***

Results of transmission electron microscopic (TEM) study of niosomal gel prepared from FG2 and FG3 formulations were shown in Figures. Most of the vesicles were well identified, spherical and discrete with sharp boundaries having large internal aqueous space. (A.Mansroi et al., 2008).The results were shown in Figure 15A-D.

***Particle size analysis***

The particle size analysis of the lornoxicam loaded niosomal gel FG2 (131 nm) and FG3 (137 nm). The obtained low value of polydispersity index lornoxicam loaded niosomal gel indicated a limited variation in particle size. (P.Loan Honeywell- Nguyen et al., 2002)(Mohamed Nasr et al., 2009).The results were shown in Figure 16A and16B.

***In-vitro release studies***

In vitro release studies were performed for the niosomal gel by dialysis bag method. The cumulative percentage drug release of formulation containing (FG1) lornoxicam plain gel showed 95.68% at 12 hours. The cumulative percentage drug release of formulation containing (FG2) span 60 niosomal gel showed 69.92% at 12 hours. The cumulative percentage drug release of formulation containing (FG3) niosomal gel showed 85.57% at 12 hours. The results showed prolonged drug release in the order FG2 > FG3 > FG1. (Kandasamy Ruckmani et al., 2010). The results were shown in Table 27 and Figure 17.

***Kinetics of drug release***

Linear regression analysis for the release was done to determine the proper order of drug release. All the formulations follow zero order kinetics. Calculation of Higuchi correlation coefficient confirms that the drugs release was proportional to the square root of time indicating that lornoxicam release from niosomal gel was diffusion controlled. The formulations FG1 (0.9957), FG2 (0.9805), FG3 (0.9964) were followed the Higuchi diffusion controlled model (Tamizharasi Sengodan *et al*., 2009).The results were shown in Table 28 Figure 18.

***Anti- inflammatory studies***

The anti-inflammatory studies were performed in albino rats by rat hind paw oedema method.

The percentage reduction in paw oedema of lornoxicam plain gel was found to be 39.64% up to 24 hour. The percentage reductions in paw oedema of formulations (FG2-Span 60), (FG3-Tween 60) were found to be 22.36% and 40.11% upto 24 hour respectively. From the above results, (FG2) span 60 niosomal gel showed sustained as well as prolonged action when compared with the Lornoxicam plain gel (FG1) and Tween 60 niosomal gel (FG3).

The formulation (FG2) Span 60 niosomal gel showed sustained release due to slower diffusion of drug into the skin and creation of reservoir effect for drug in the skin. The other components of niosomes (surfactant, cholesterol) also deposit along with drug into the skin and thereby increasing the drug retention capacity into skin (Munish Ahuja *et al*., 2011). The results were shown in Table 29 and Figure 19.

# CHAPTER- XIII

# SUMMARY AND CONCLUSION

- The $\lambda_{max}$ of Lornoxicam was found to be 375nm in phosphate buffer saline (PBS) pH 7.4.
- The Lornoxicam obeys the Beers law within the concentration of 2 to 20 µg/ml.
- DSC and FT-IR studies indicated that there was no interaction between drug and excipients.
- Lornoxicam niosomes were prepared using different ratios of non-ionic surfactants with cholesterol and same concentration of drug by thin film hydration method.
- The niosomal formulations were evaluated for drug content, entrapment efficiency, invitro release studies and kinetic drug release.
- The percentage drug content of all the formulations was found to be in the range of 95% to 96.95%.
- The entrapment efficiency was found to be higher for niosomal formulation F32 when compared to other niosomal formulations due to the length of alkyl chain of Brij 52.
- The invitro release study showed prolonged release profile for all the thirty two niosomal formulations compared to the pure drug solution. The formulation F24 showed highest drug release at $12^{th}$ hour. The formulation F8 showed controlled drug release at $12^{th}$ hour.
- On the basis of entrapment efficiency and invitro release study, the niosomal formulation (F8) and (F24) equivalent to 0.02% w/w of lornoxicam was incorporated into carbopol gel base and evaluated with 1% w/w of plain lornoxicam gel. The gels were easy to prepare and found to be homogenous in composition.
- The niosomal gels were evaluated for drug content.

- The pH and viscosity of the gel formulation were also evaluated.
- The Transmission electron microscopy showed spherical and discrete vesicles with sharp boundaries having large internal aqueous phase.
- The particle size evaluated for niosomal gels FG2 and FG3 were found to be 131 nm and 137 nm.
- The invitro release studies of lornoxicam niosomal gel formulations exposed that the release rate of lornoxicam niosomal gel(FG2) was slower than that of plain lornoxicam gel(FG1) and Tween 60 niosomal gel(FG3).
- The invitro release kinetics also evaluated that all niosomal formulations obeys the Higuchi diffusion model.
- The anti-inflammatory activity of lornoxicam niosomal gel (FG2) showed uniform and prolonged activity when compared to the plain gel (FG1) and Tween 60 niosomal gel (FG3). Therefore the present study suggests the potentiality of niosomal gel to enhance the therapeutic effect and minimize the side effects of lornoxicam.
- From the above study it is concluded that the niosomes can be considered as promising drug delivery vehicles which increase the duration of the drugs. Thus niosomes help in formulating dosage forms having more prolonged action.
- The niosomal delivery of lornoxicam in carbopol gel base acts as a suitable topical drug delivery system which may be given safely to all patients suffering from rheumatoid arthritis and osteoarthritis. The stability of niosomes improved after incorporation into gel base may be due to prevention of fusion of niosomes. Further the pharmacokinetic and other clinical studies may be carried out in future.

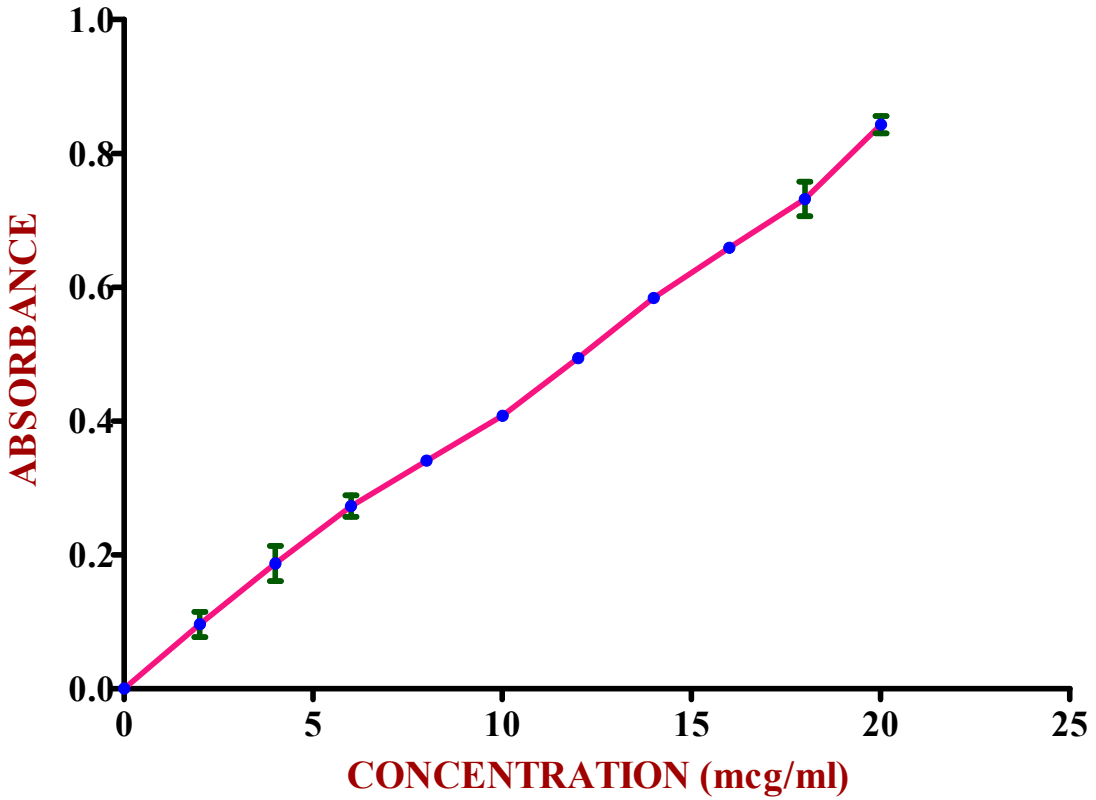
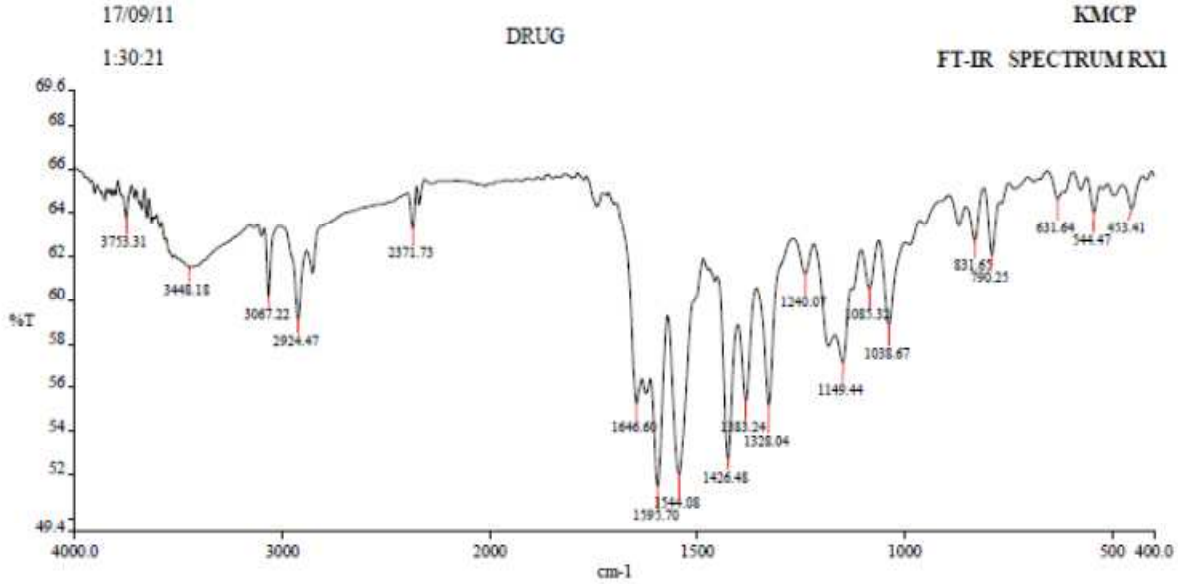
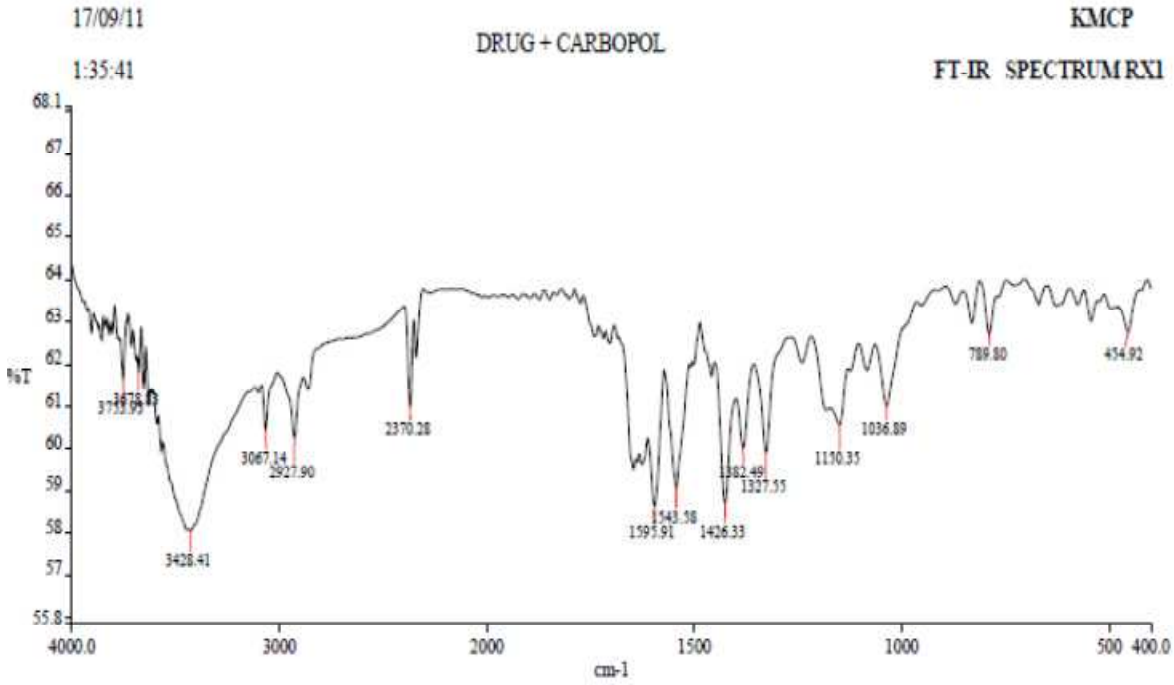



**Figure.8 STANDARD CURVE OF LORNOXICAM IN PBS pH 7.4**

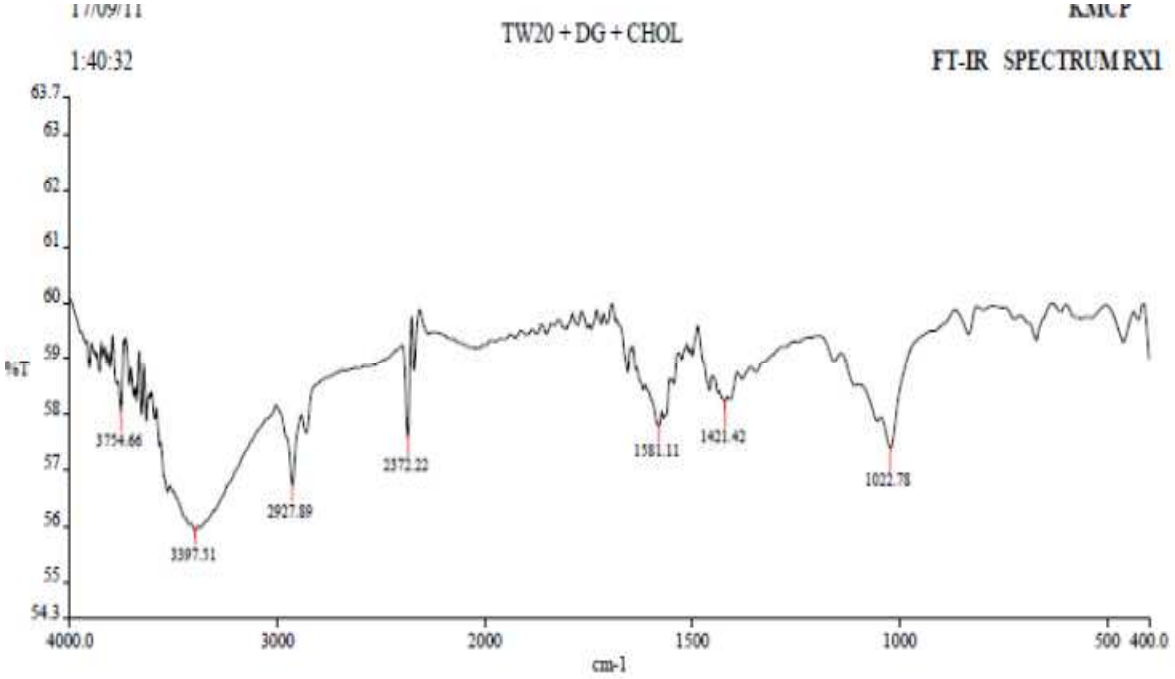
**A) LORNOXICAM**



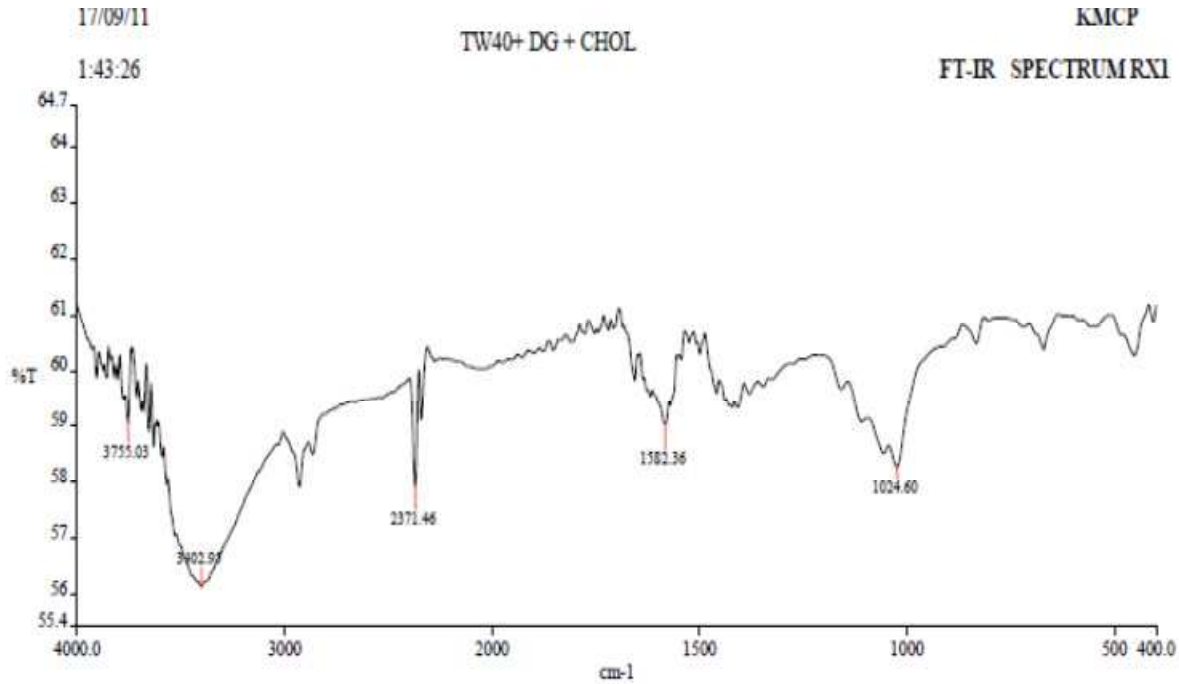

**B) LORNOXICAM AND CARBOPOL**



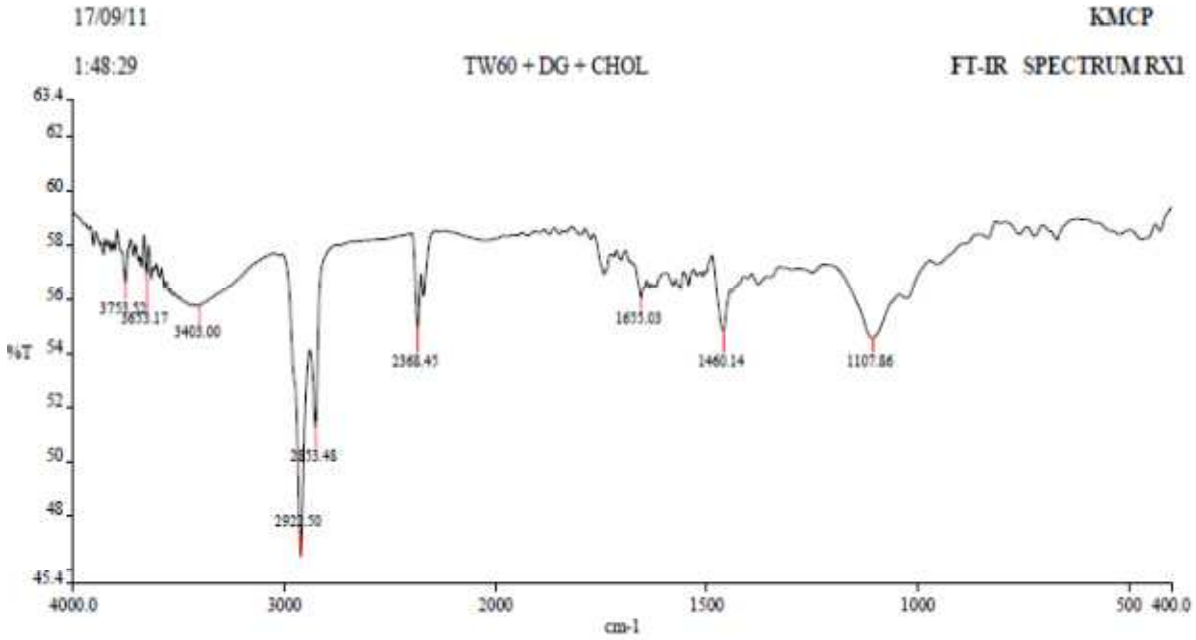
## C) LORNOXICAM, TWEEN 20 AND CHOLESTEROL



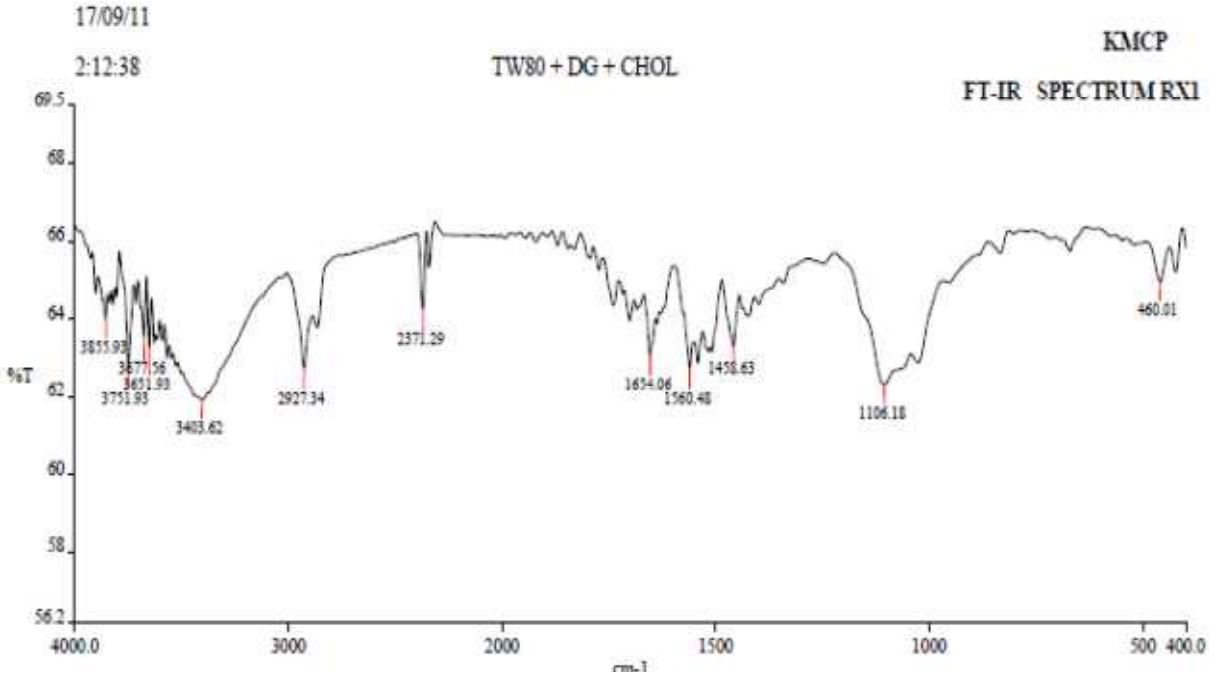

## D) LORNOXICAM, TWEEN 40 AND CHOLESTEROL



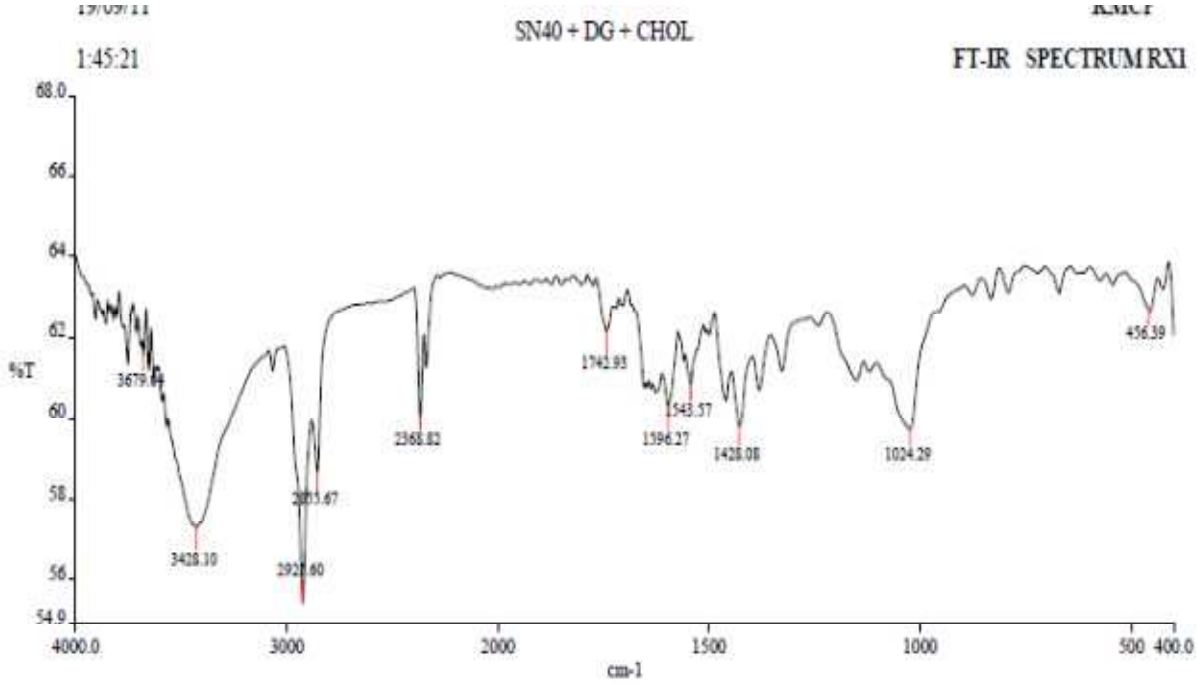
## E) LORNOXICAM, TWEEN 60 AND CHOLESTEROL



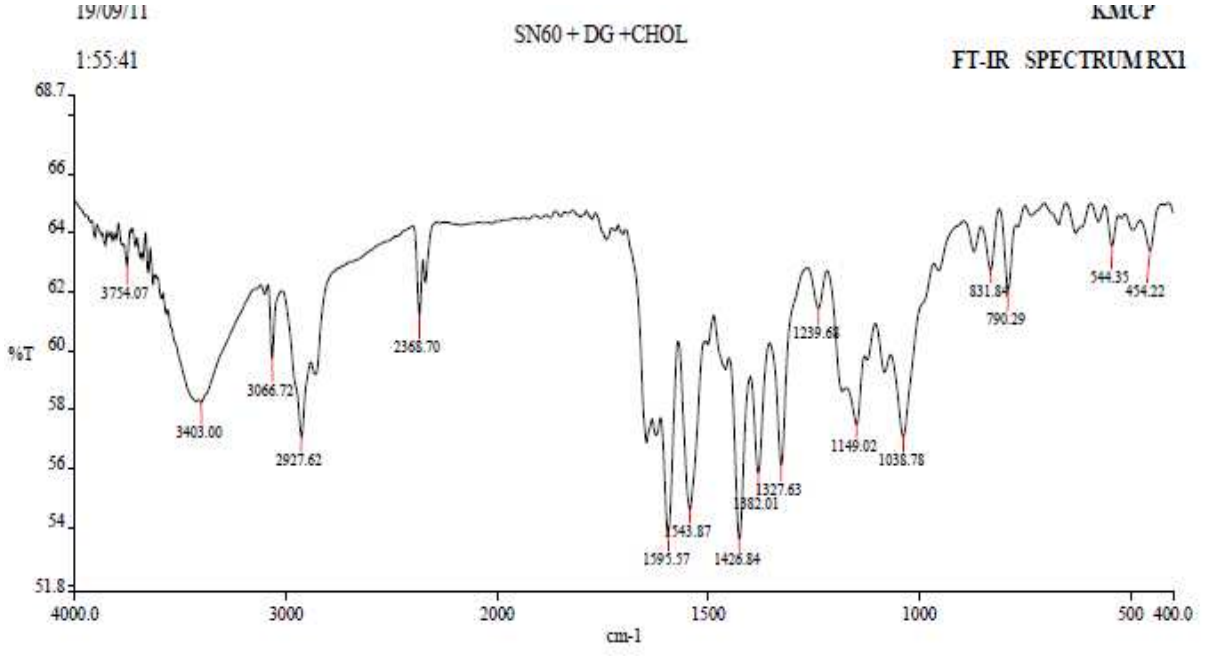

## F) LORNOXICAM, TWEEN 80 AND CHOLESTEROL



## G) LORNOXICAM, SPAN 40 AND CHOLESTEROL




## H) LORNOXICAM, SPAN 60 AND CHOLESTEROL



**I) LORNOXICAM, SPAN 80 AND CHOLESTEROL**

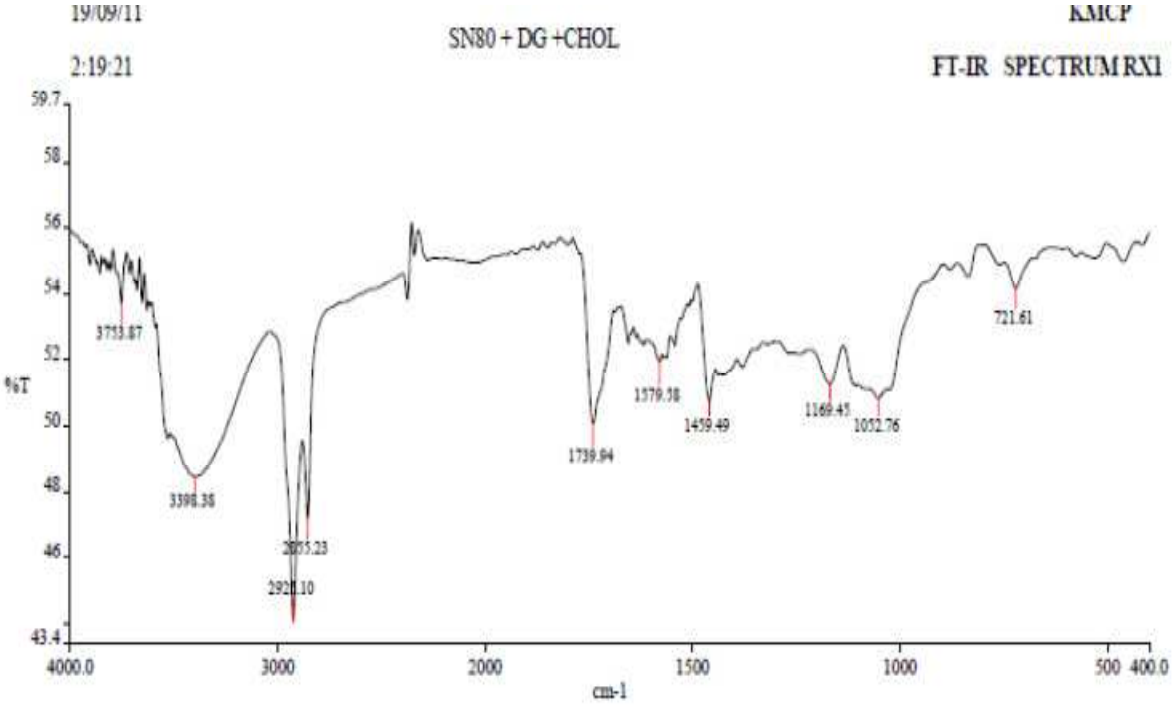
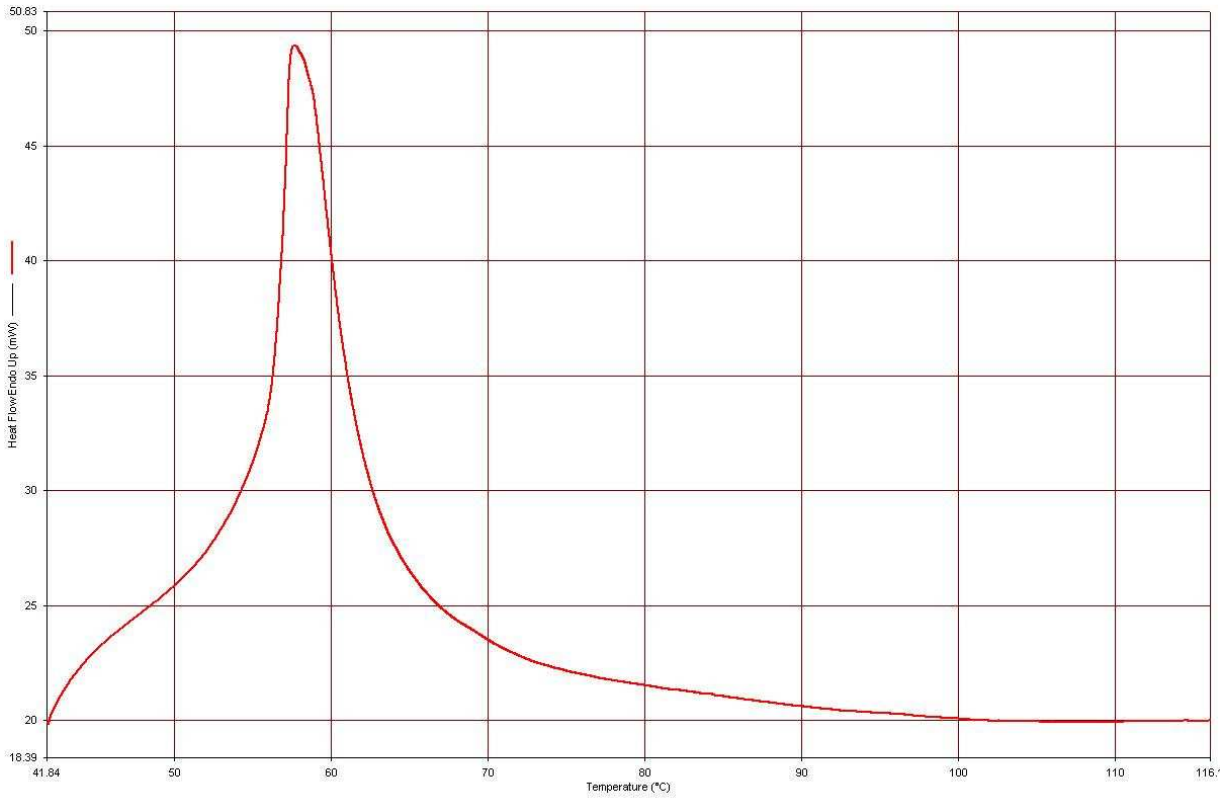
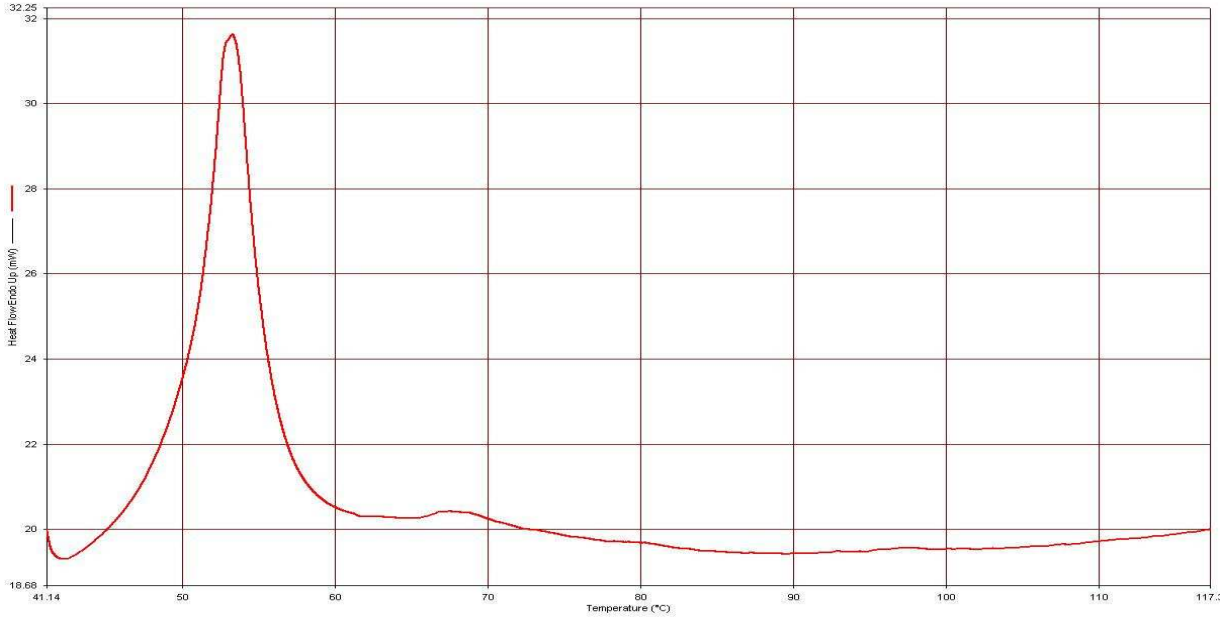



**Figure.9 FT-IR SPECTRUM ARE AS FOLLOWS: A) LORNOXICAM, B) LORNOXICAM AND CARBOPOL, C) LORNOXICAM, TWEEN 20 AND CHOLESTEROL, D) LORNOXICAM, TWEEN 40 AND CHOLESTEROL, E) LORNOXICAM, TWEEN 60 AND CHOLESTEROL, F) LORNOXICAM, TWEEN 80 AND CHOLESTEROL, G) LORNOXICAM, SPAN 40 AND CHOLESTEROL, H) LORNOXICAM, SPAN 60 AND CHOLESTEROL, I) LORNOXICAM, SPAN 80 AND CHOLESTEROL**

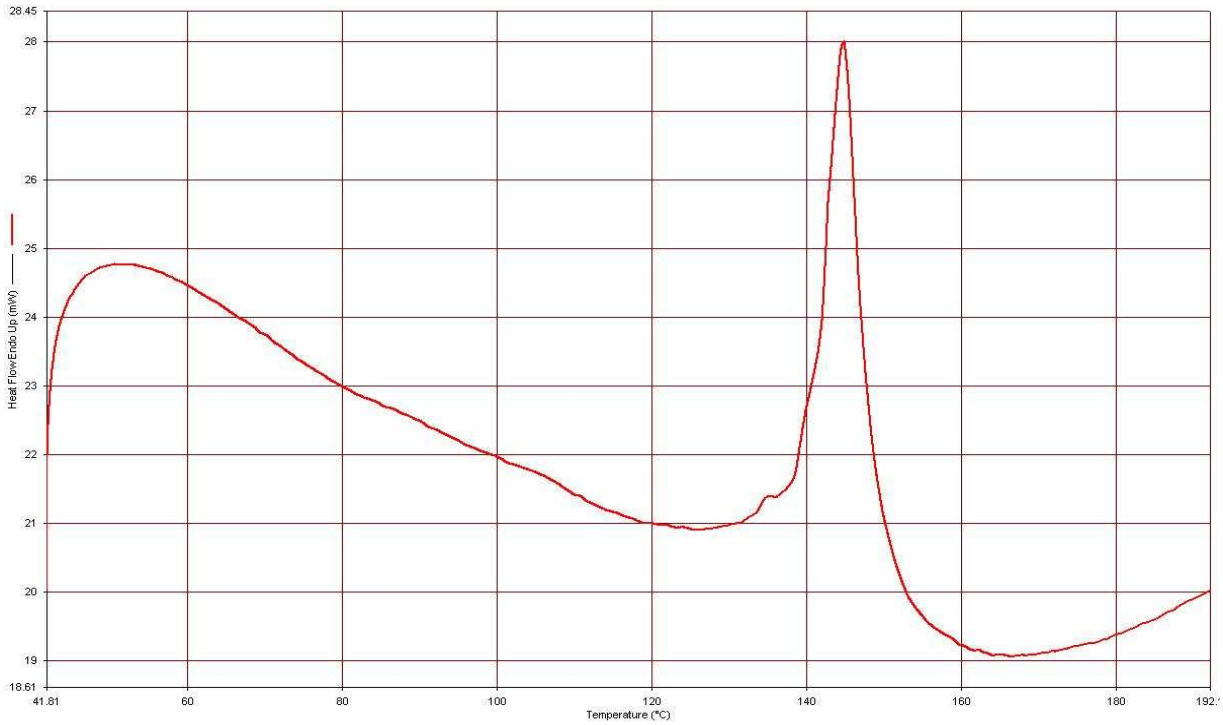
## A) CHOLESTEROL



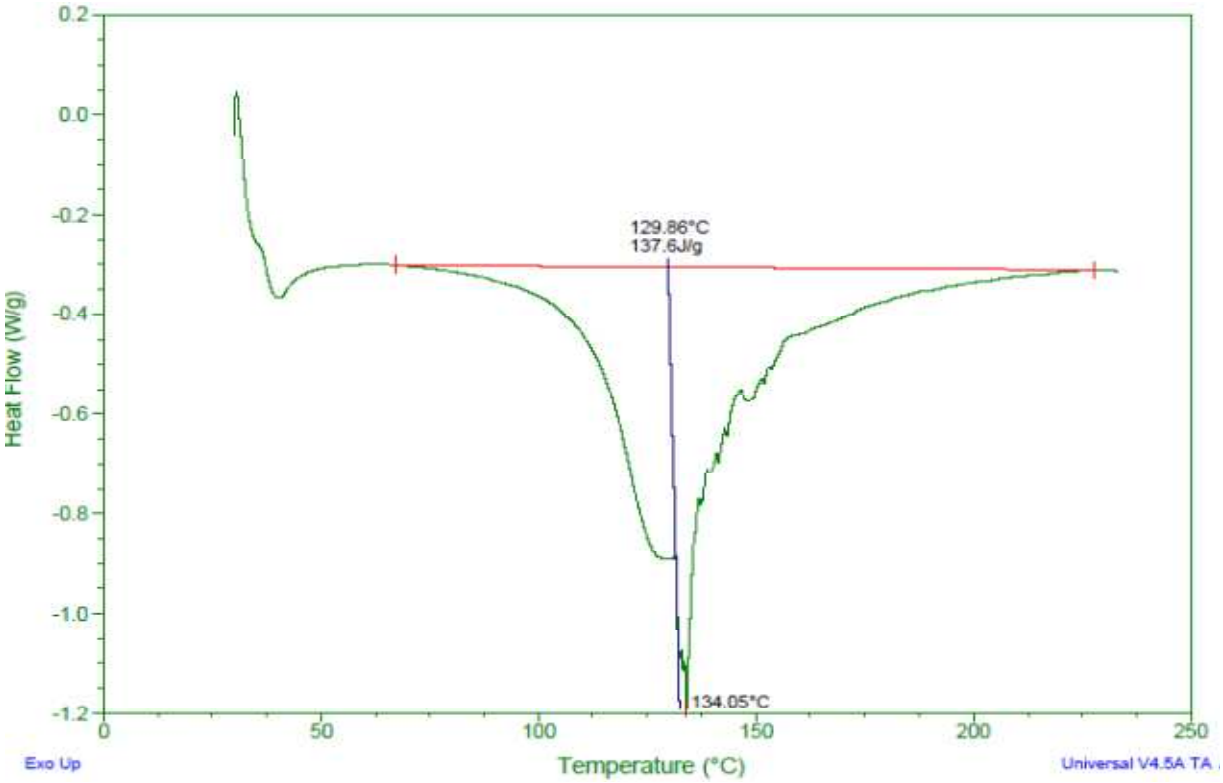

## B) SPAN-60



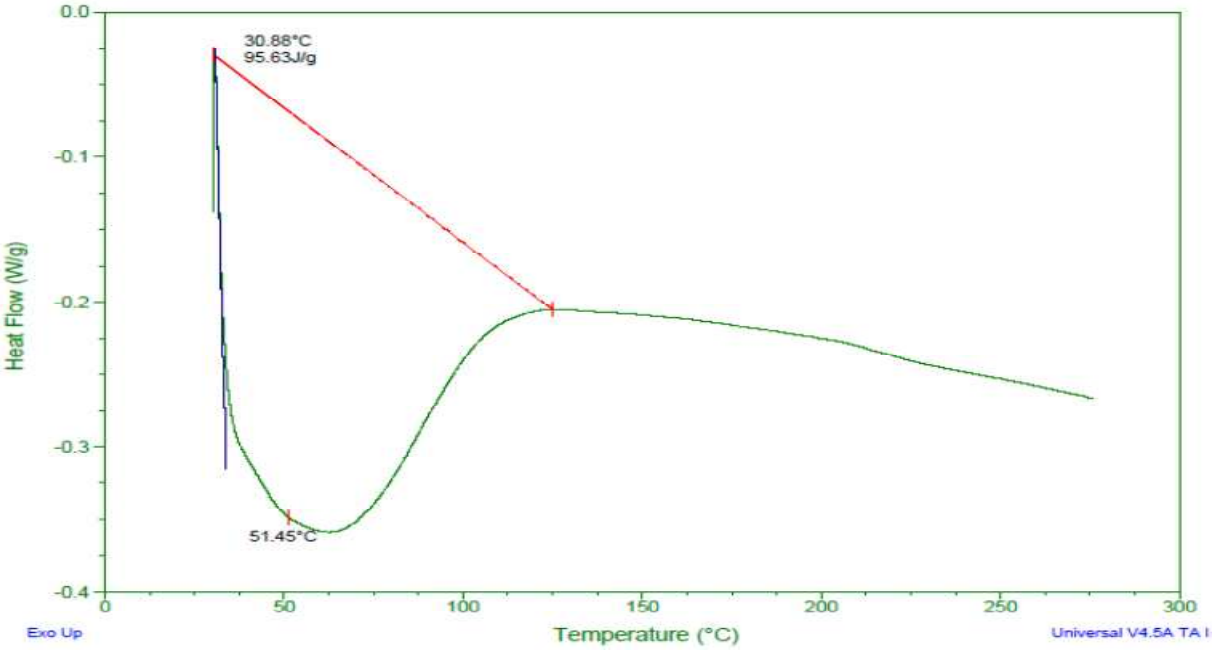
## C) SPAN- 40



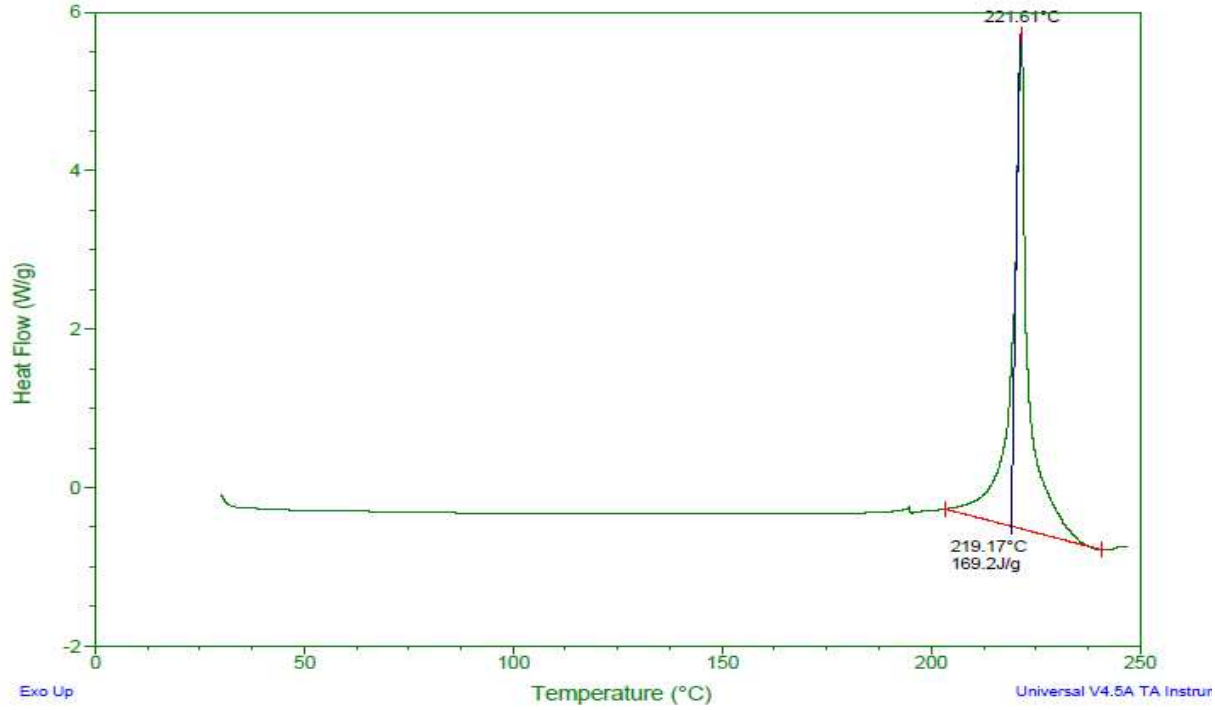

## D) TWEEN 20



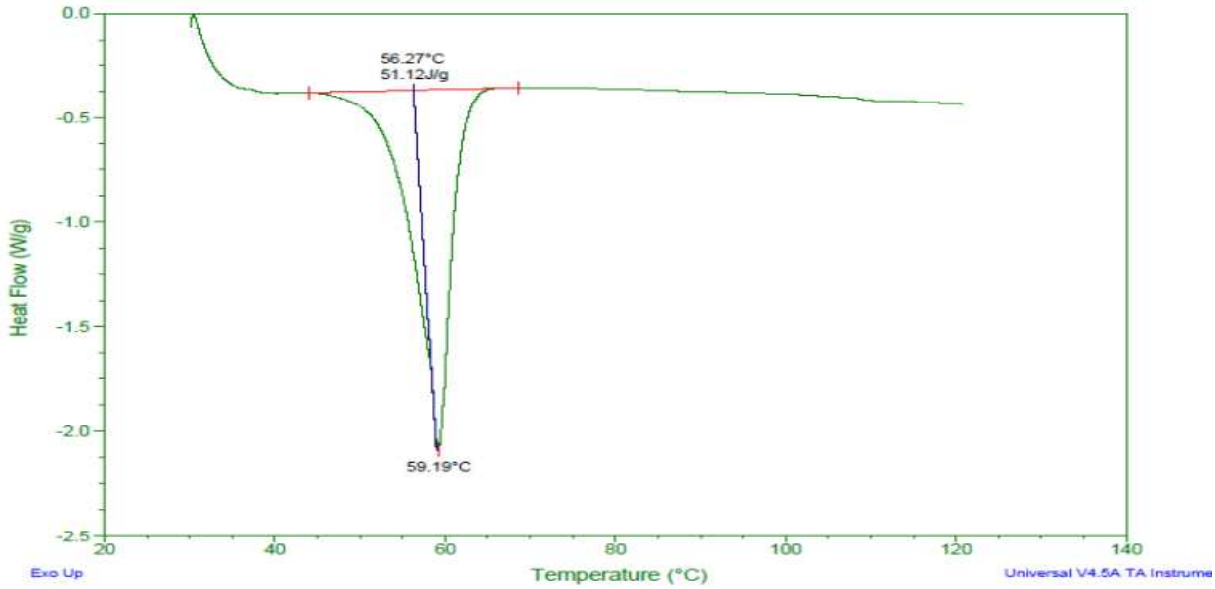
**E) TWEEN 40**



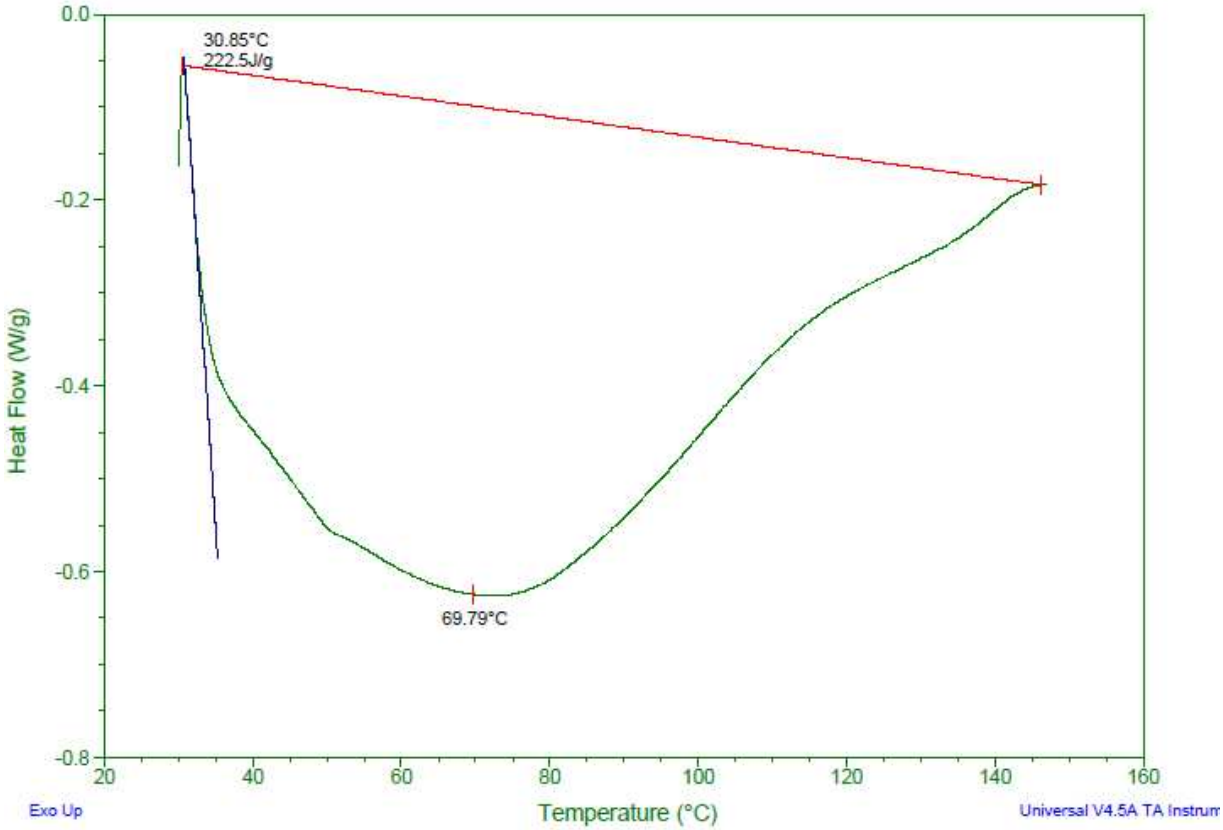

**F) LORNOXICAM**



## G) SPAN 60 LORNOXICAM CHOLESTEROL




## H) TWEEN 60 CHOLESTEROL LORNOXICAM



**I) TWEEN 80 CHOLESTEROL LORNOXICAM**

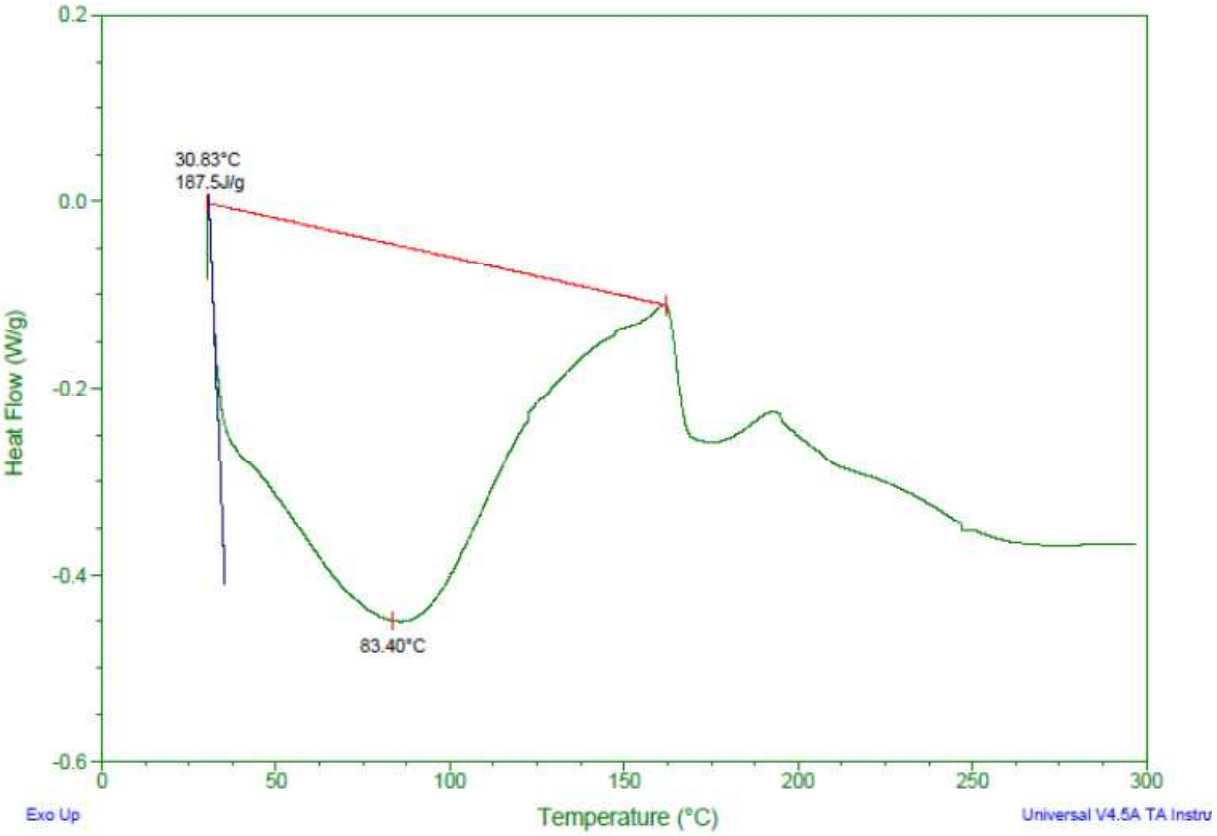



**FIGURE 10 DSC THERMOGRAM ARE AS FOLLOWS:**

**A) CHOLESTEROL B) SPAN-60 C) SPAN-40 D) TWEEN-20 E) TWEEN-40 F) LORNOXICAM G) SPAN-60 , CHOLESTEROL AND LORNOXICAM H) TWEEN-60, CHOLESTEROL AND LORNOXICAM I) TWEEN-80, CHOLESTEROL AND LORNOXICAM**

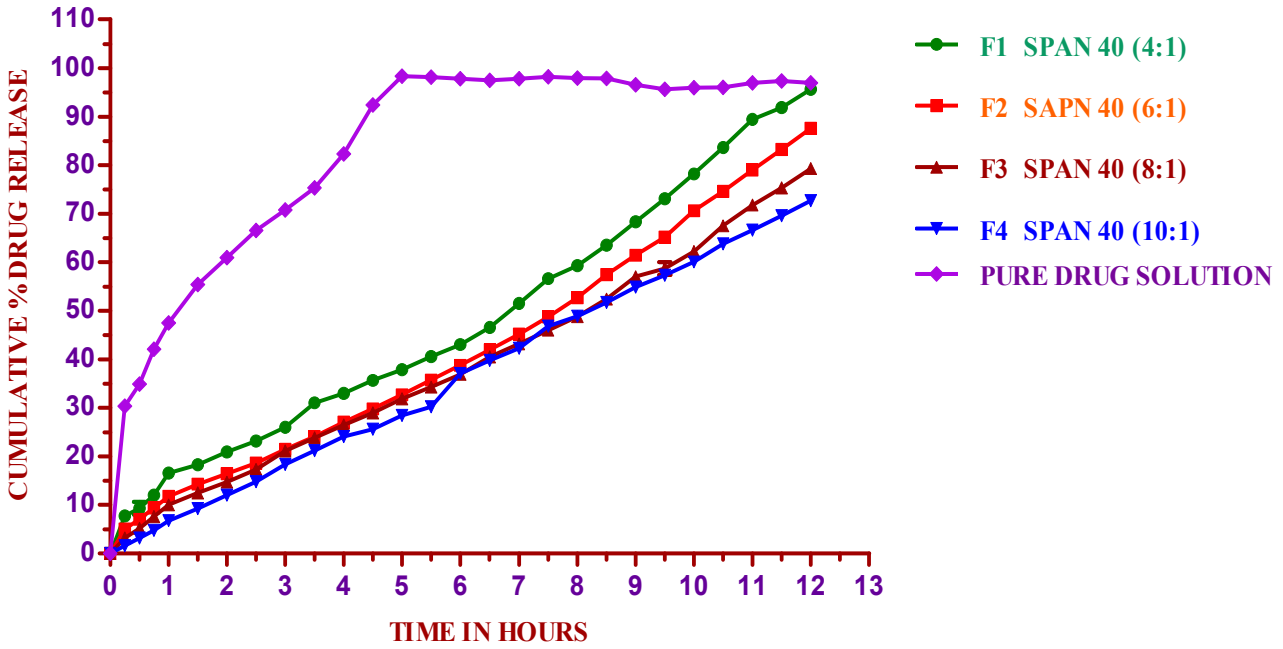



**Figure.12A COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING SPAN 40 AT DIFFERENT RATIOS**

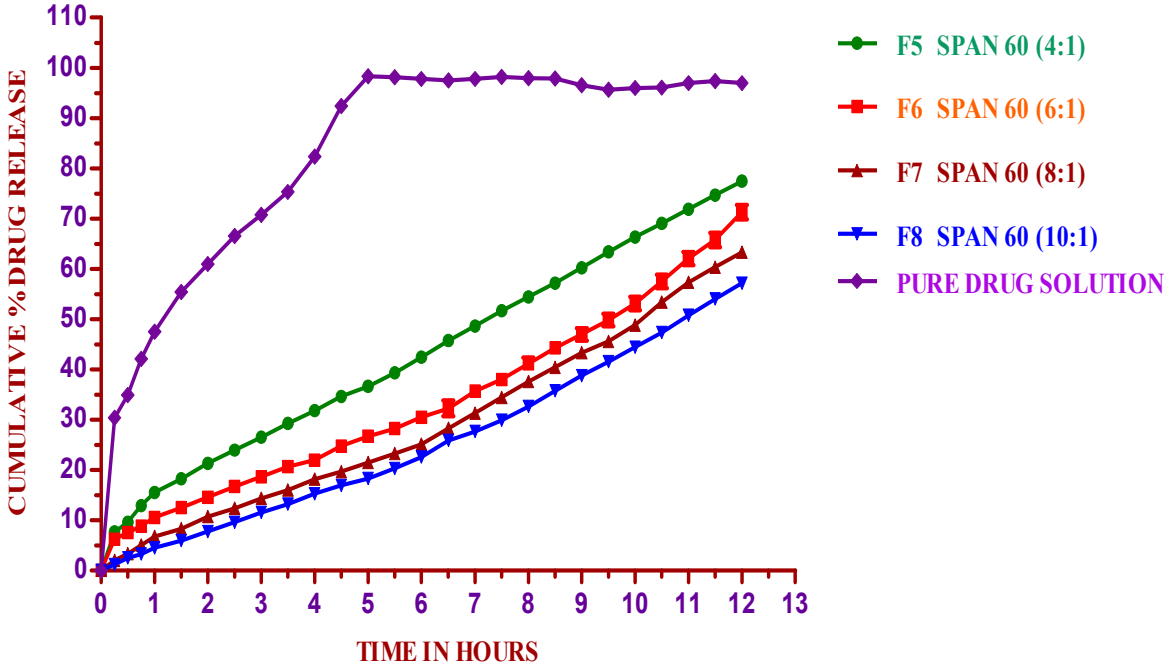



**Figure.12B COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING SPAN 60 AT DIFFERENT RATIOS**

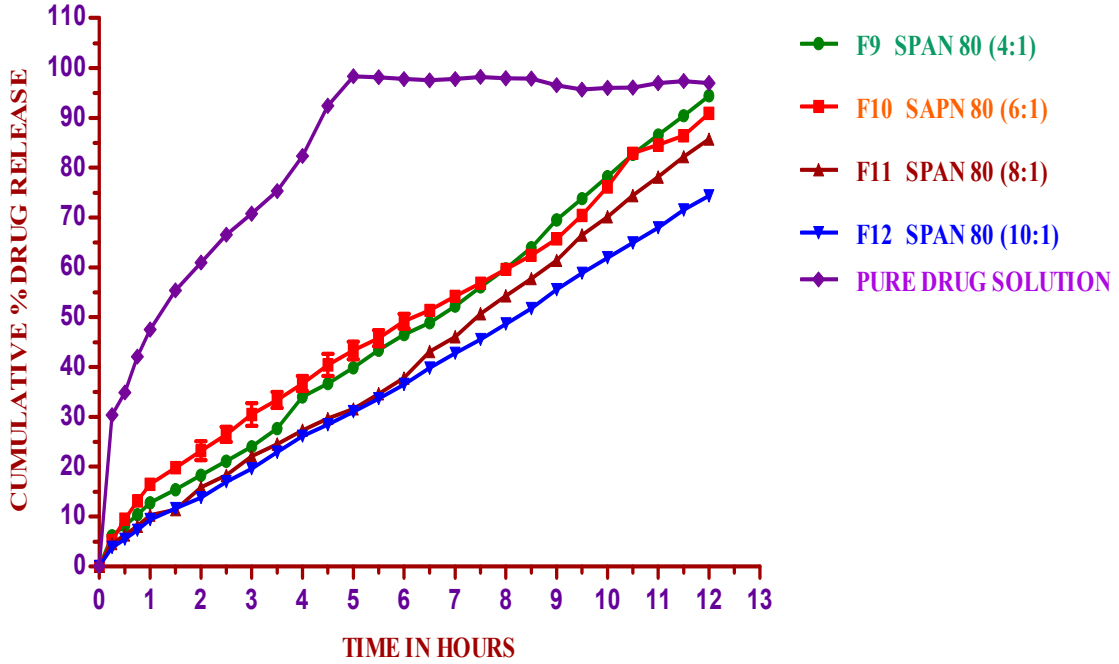



**Figure.12C COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING SPAN 80 AT DIFFERENT RATIOS**

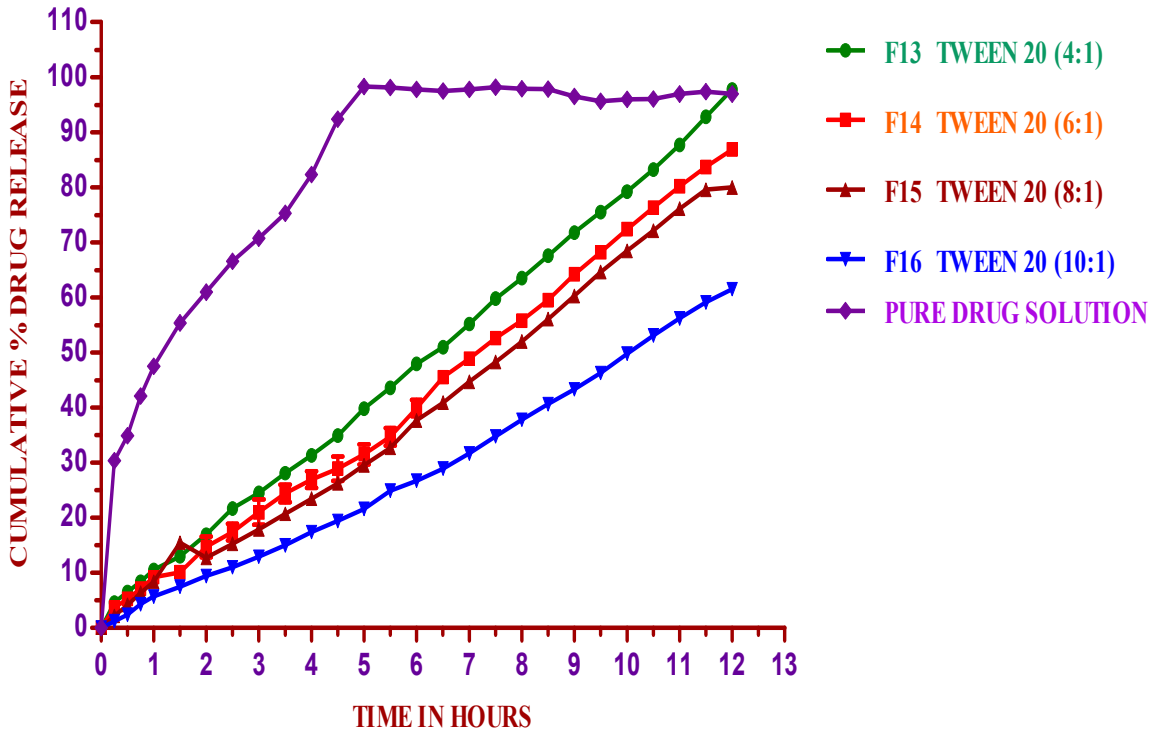



**Figure.12D COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING TWEEN 20 AT DIFFERENT RATIOS**

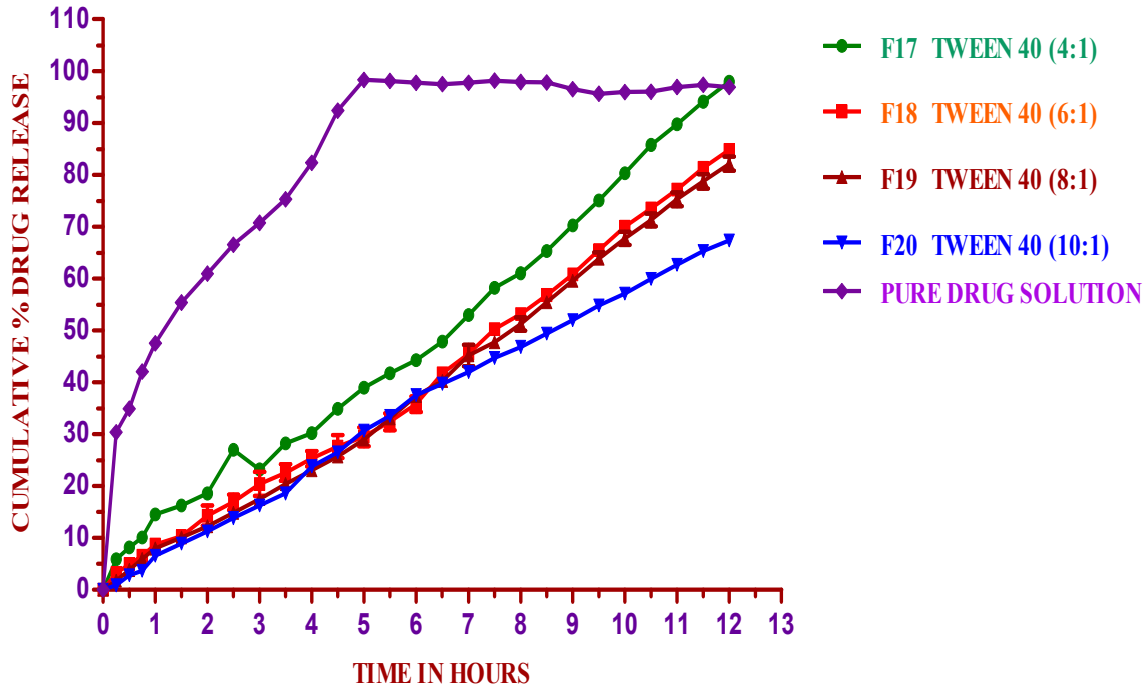



**Figure.12E COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING TWEEN 40 AT DIFFERENT RATIOS**

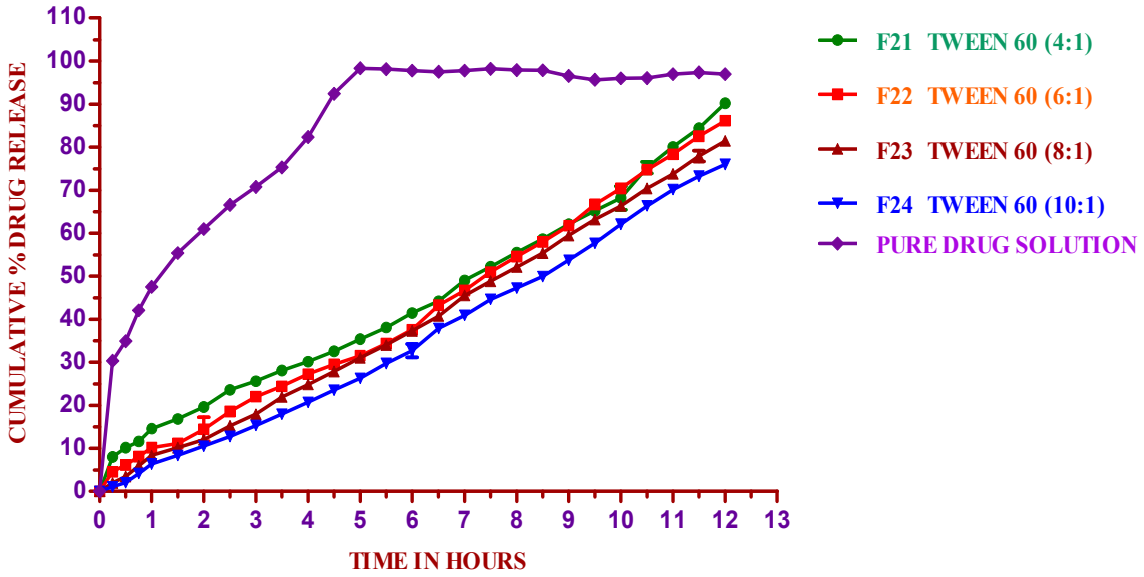



**Figure.12F COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING TWEEN 60 AT DIFFERENT RATIOS**

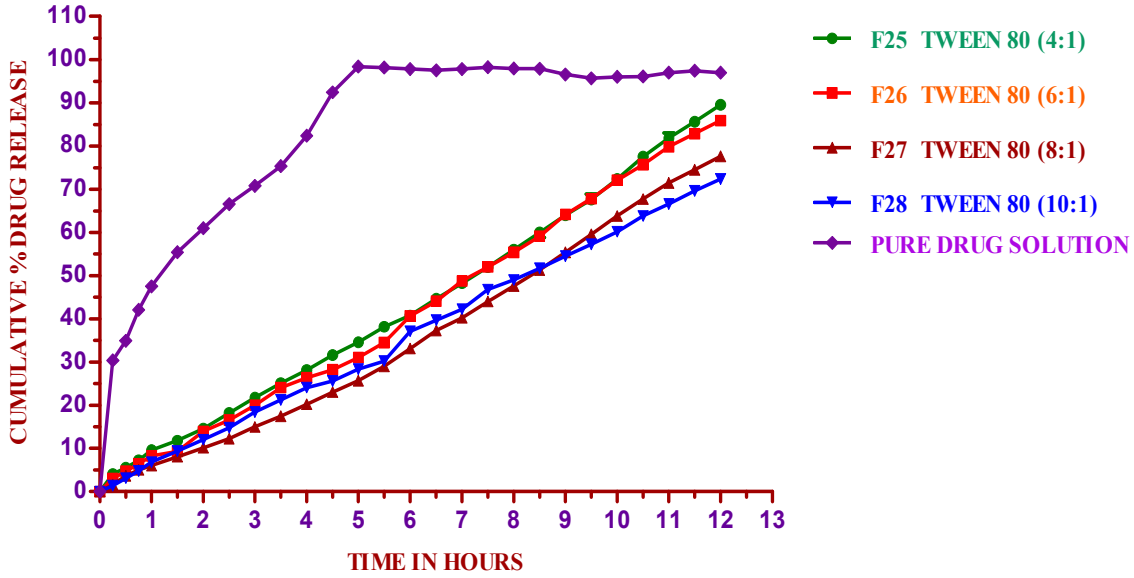



**Figure.12G COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING TWEEN 80 AT DIFFERENT RATIOS**

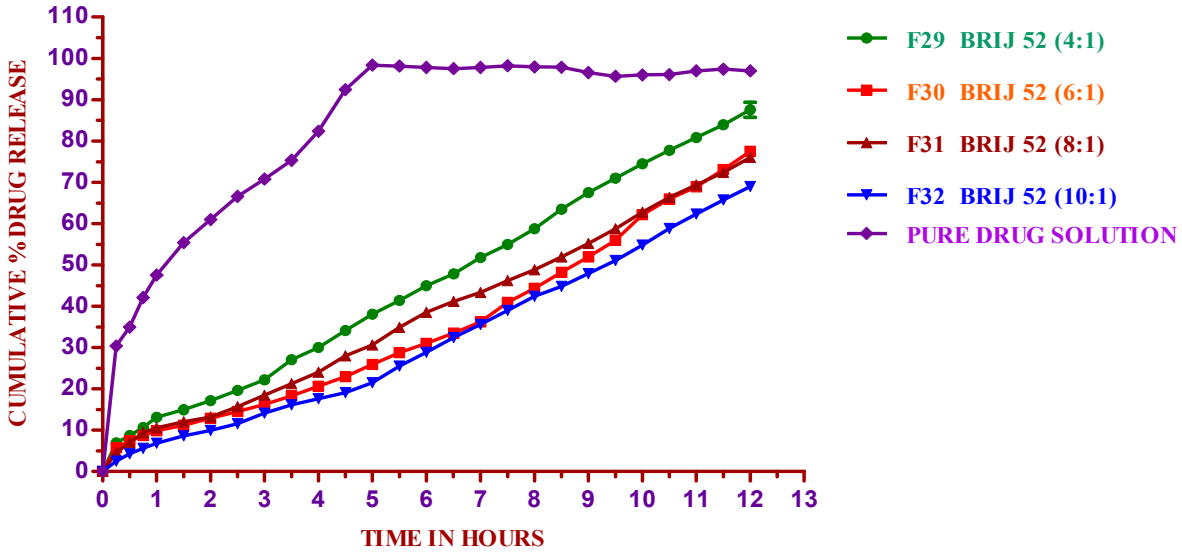



**Figure.12H COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING BRIJ 52 AT DIFFERENT RATIOS**

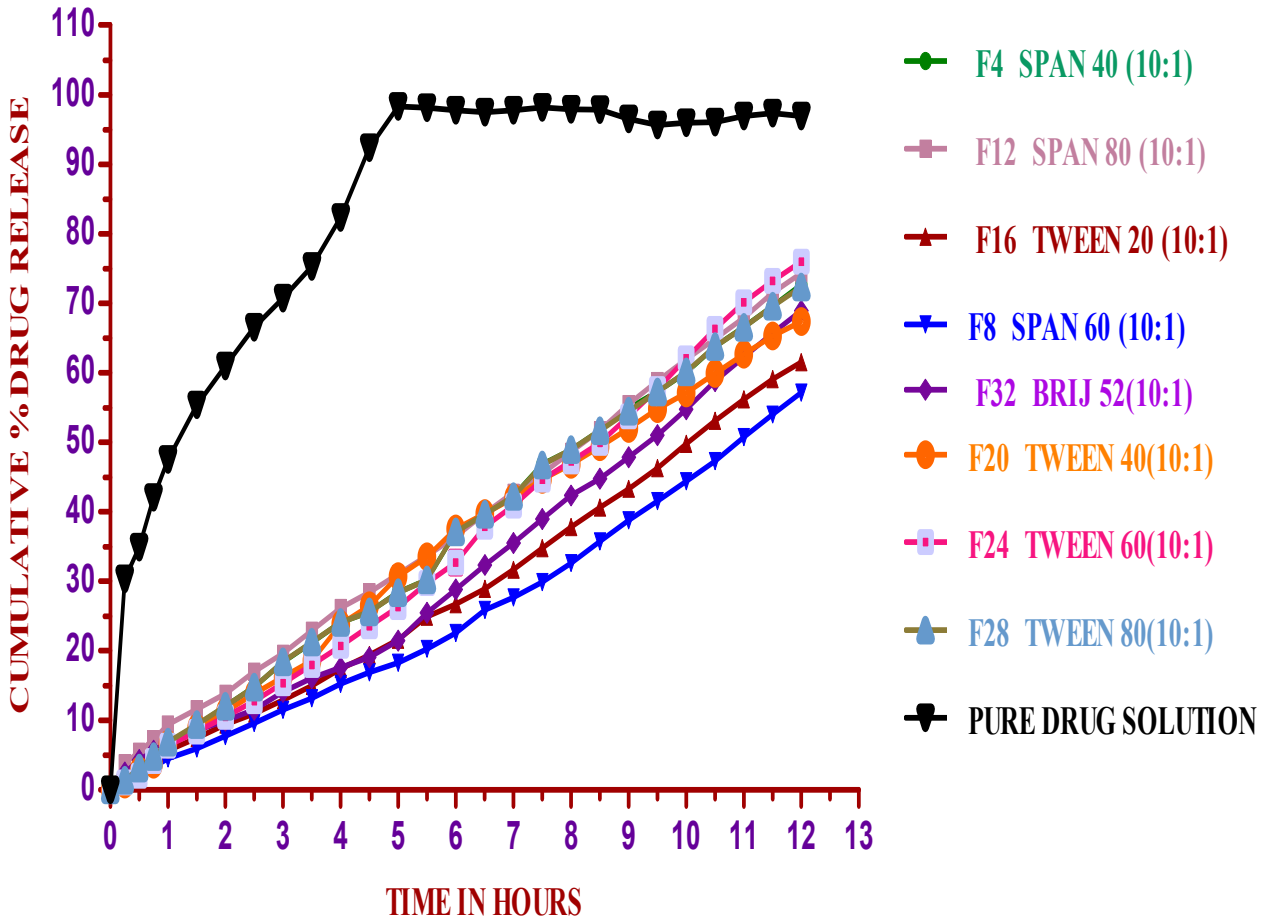



**Figure.13 COMPARISON OF INVITRO RELEASE OF LORNOXICAM NIOSOMES CONTAINING DIFFERENT SURFACTANTS AT 10:1 RATIOS**

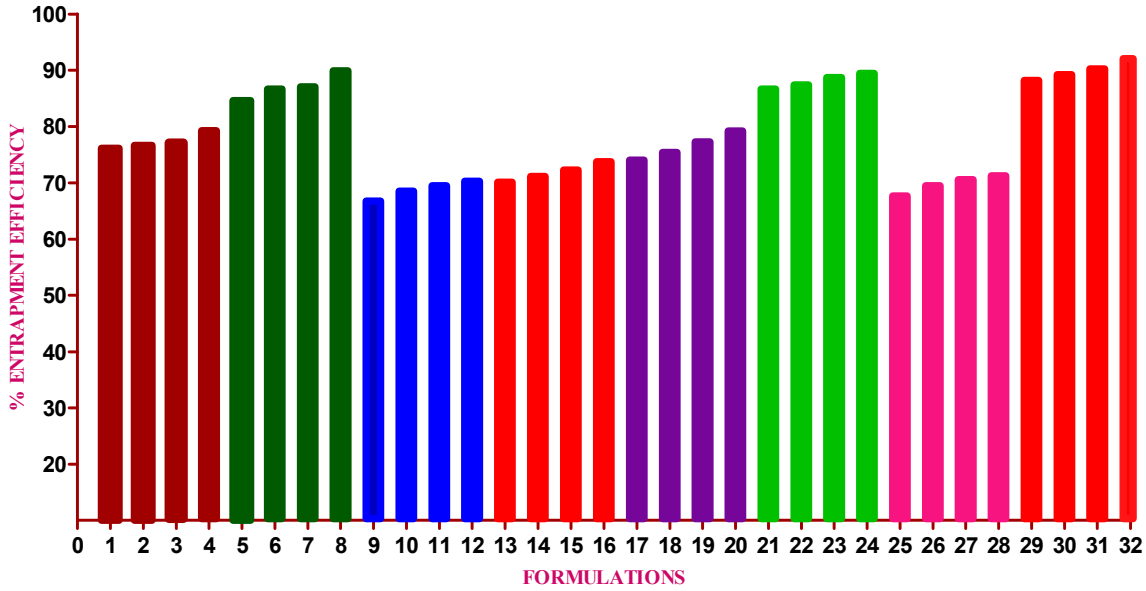
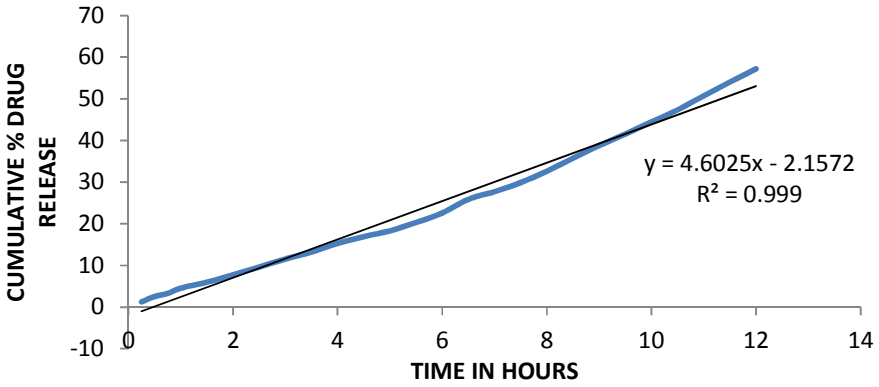
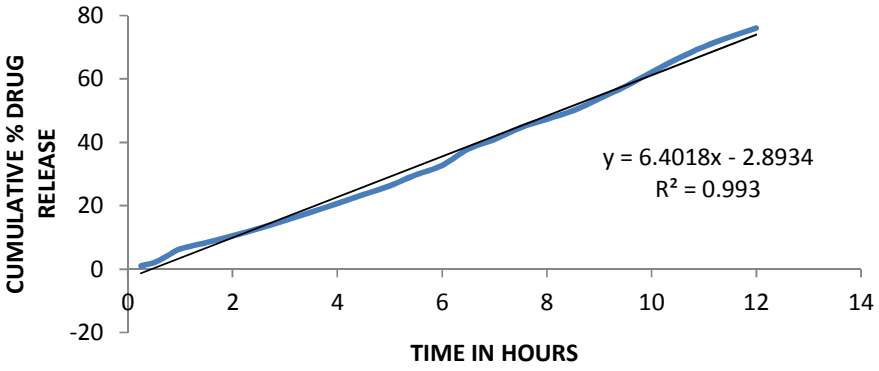
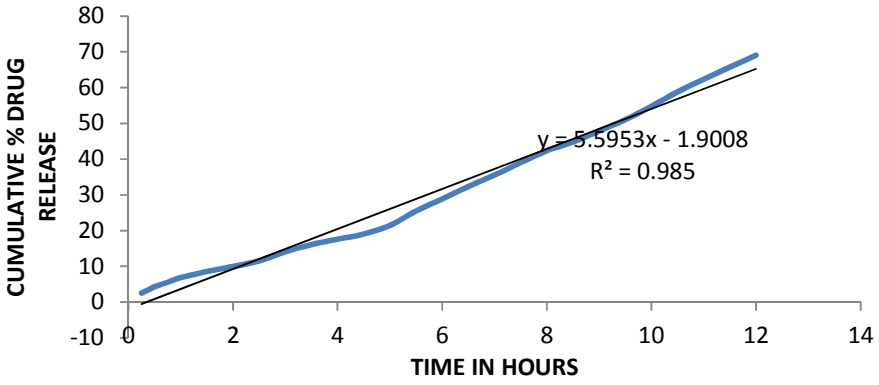



**Figure.11 COMPARISON OF PERCENTAGE ENTRAPPMENT EFFICIENCIES OF FORMULATIONS CONTAINING DIFFERENT NON-IONIC SURFACTANTS**

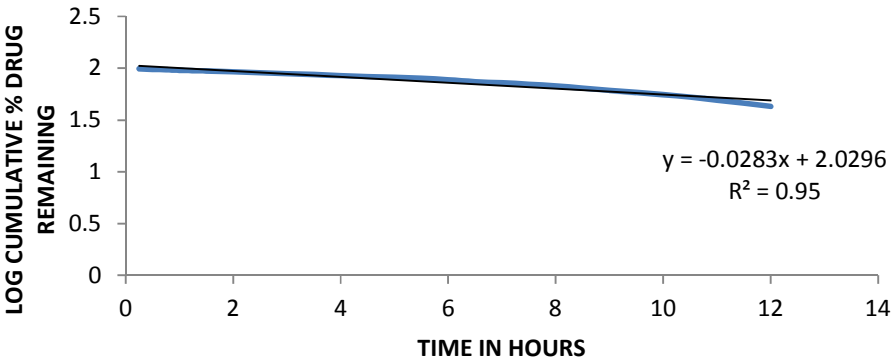
## A) ZERO ORDER

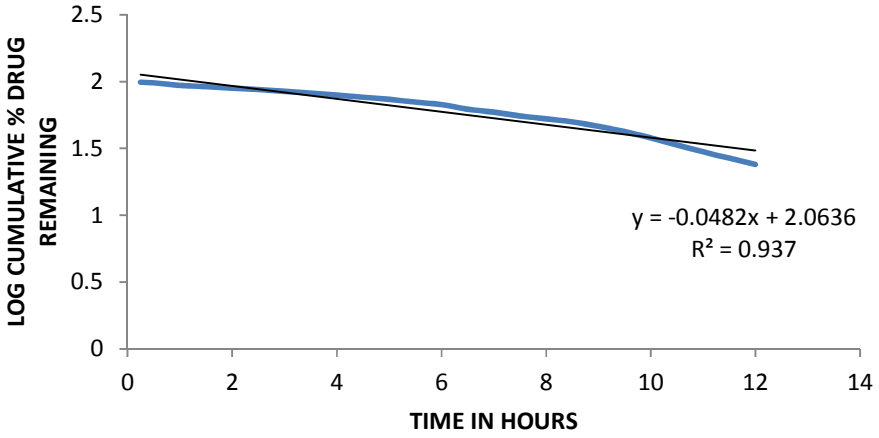

CUMULATIVE % DRUG RELEASE
70
60
50
40
30
20
10
0
-10
0
2
4
6
8
10
12
14
TIME IN HOURS
y = 4.6025x - 2.1572
R² = 0.999

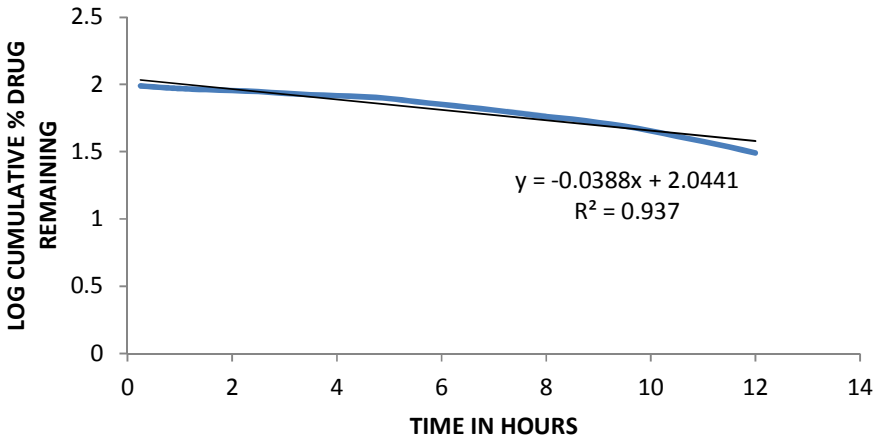

CUMULATIVE % DRUG RELEASE
80
60
40
20
0
-20
0
2
4
6
8
10
12
14
TIME IN HOURS
y = 6.4018x - 2.8934
R² = 0.993


CUMULATIVE % DRUG RELEASE
80
70
60
50
40
30
20
10
0
-10
0
2
4
6
8
10
12
14
TIME IN HOURS
y = 5.5953x - 1.9008
R² = 0.985

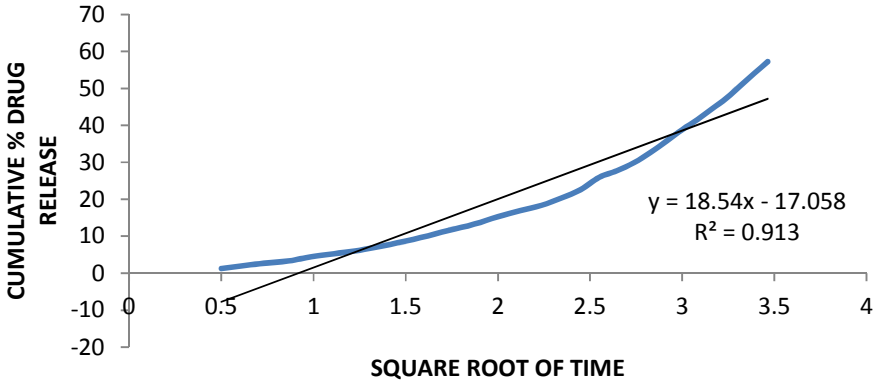
## B) FIRST ORDER

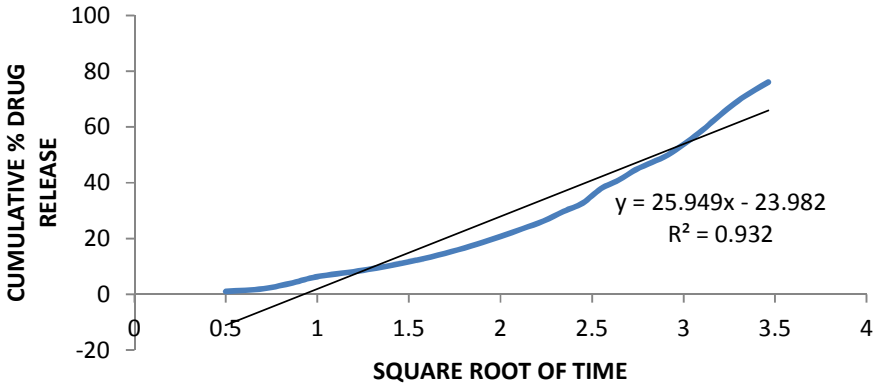


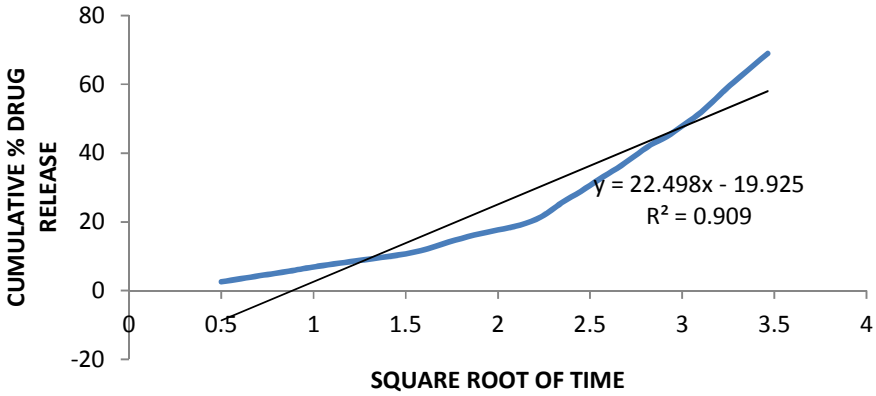



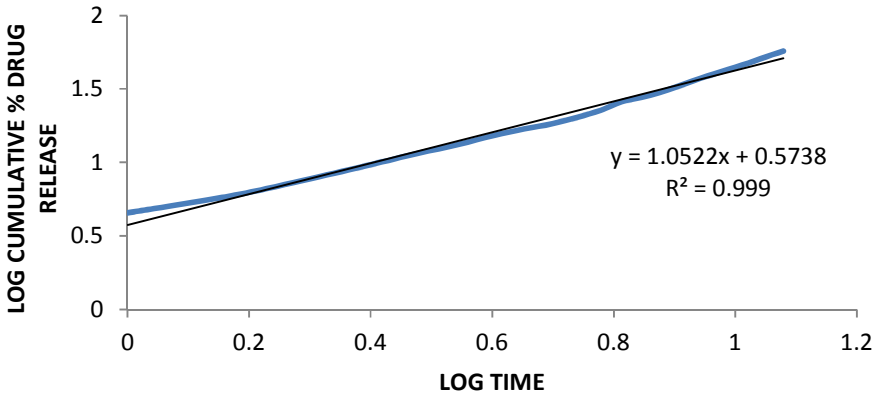
## C) HIGUCHI RELEASE KINETICS

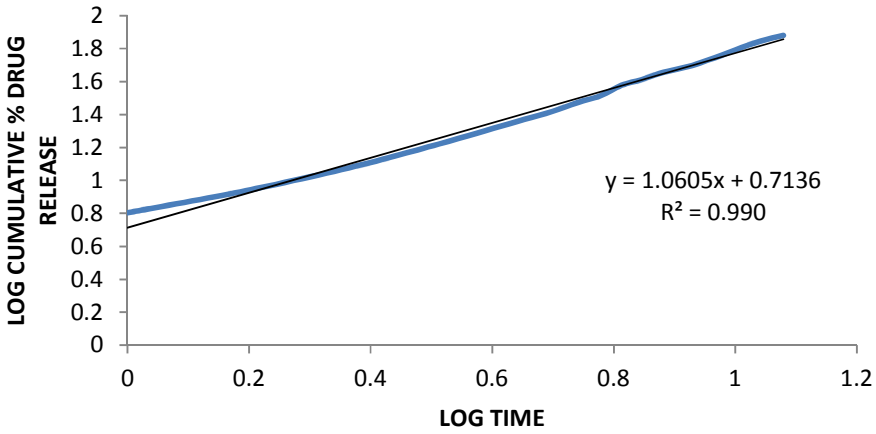


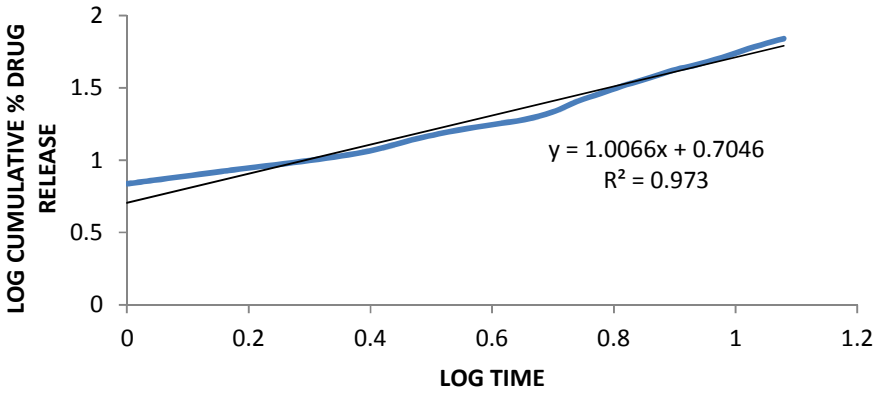



## D) KORSMEYER PEPPAS MODEL



**E) HIXON CROWELL**

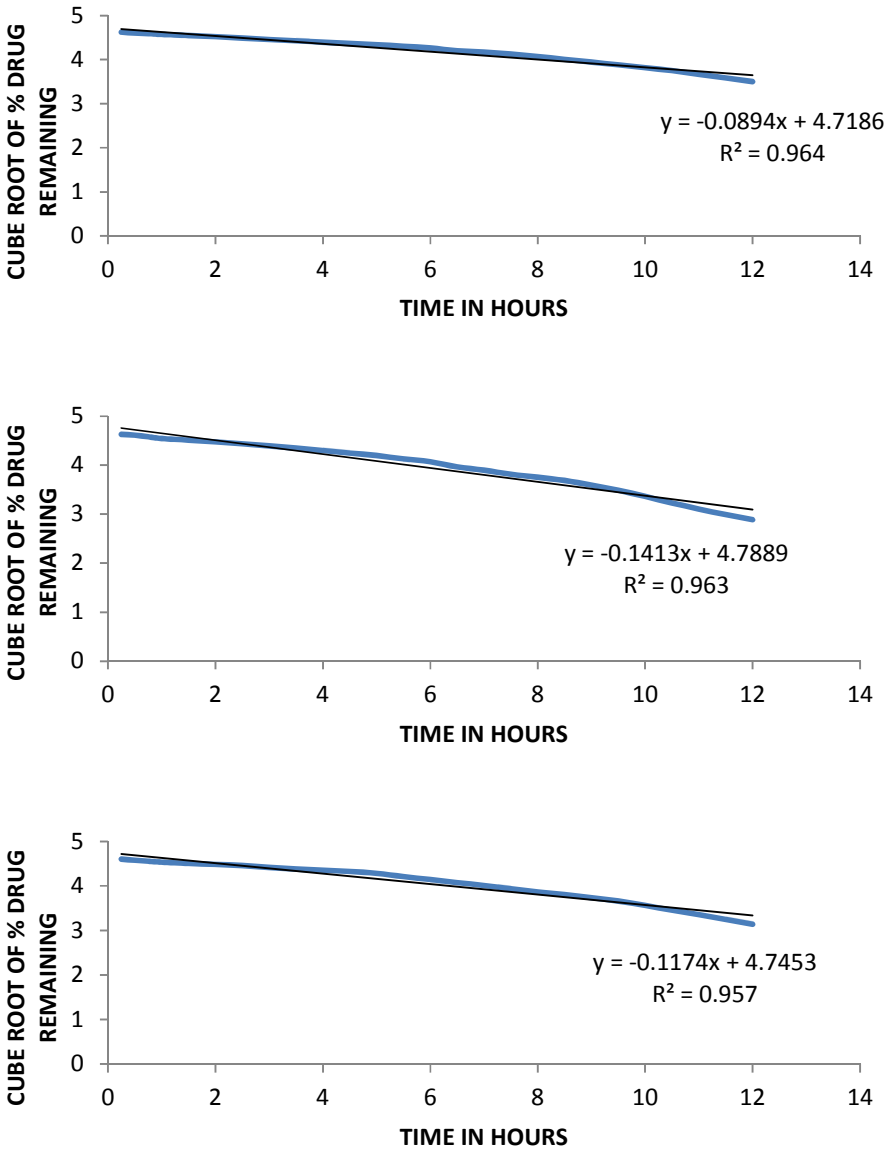



**Figure.14 COMPARISON OF INVITRO RELEASE KINETICS OF FORMULATIONS F8 (SPAN 60), F24 (TWEEN 60) AND F32 (BRIJ 52) AS FOLLOWS A) ZERO ORDER B) FIRST ORDER C) HIGUCHI RELEASE KINETICS D) KORSMEYER PEPPAS MODEL E) HIXON CROWEL**

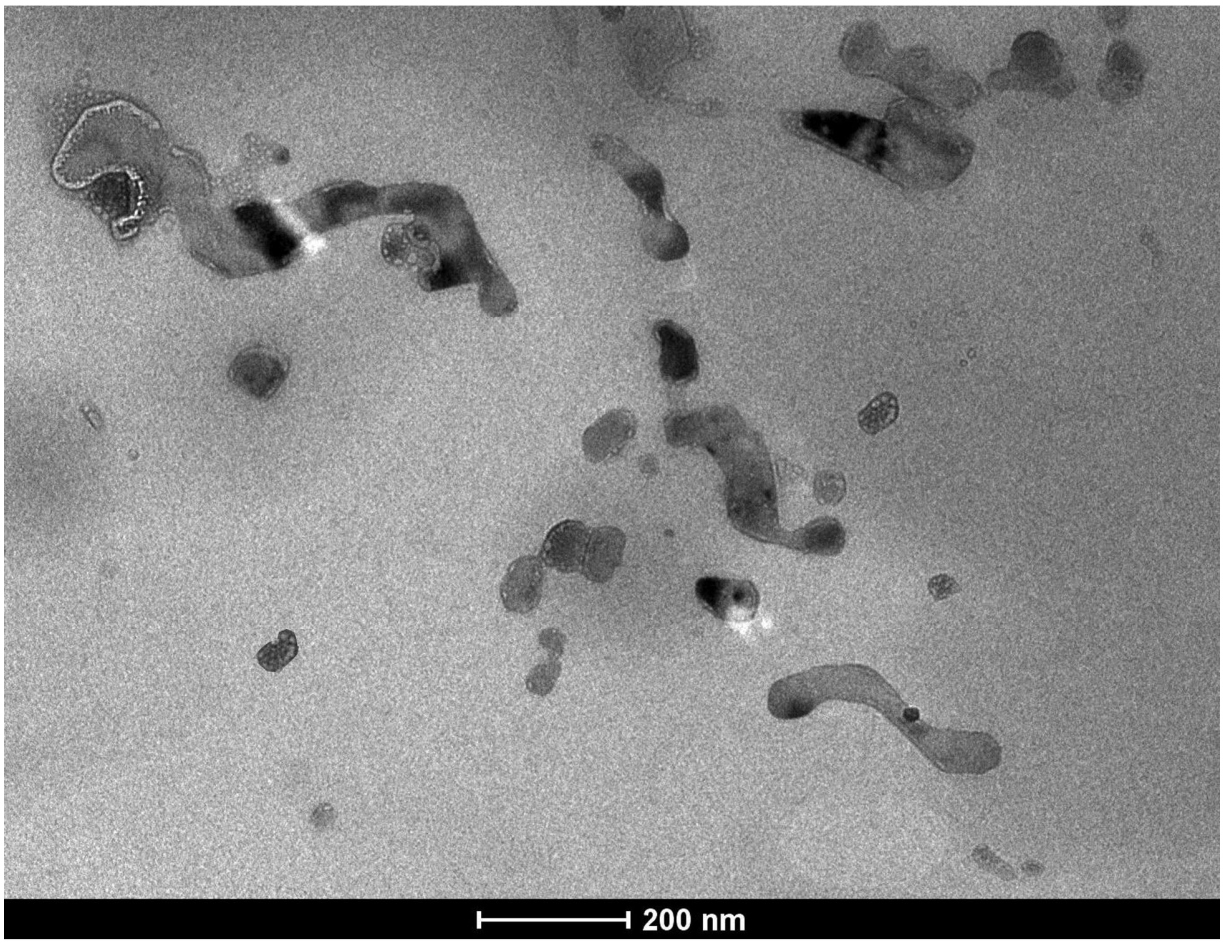



**Figure.15A TEM IMAGE OF NIOSOMAL GEL FORMULATIONS (FG 2)**

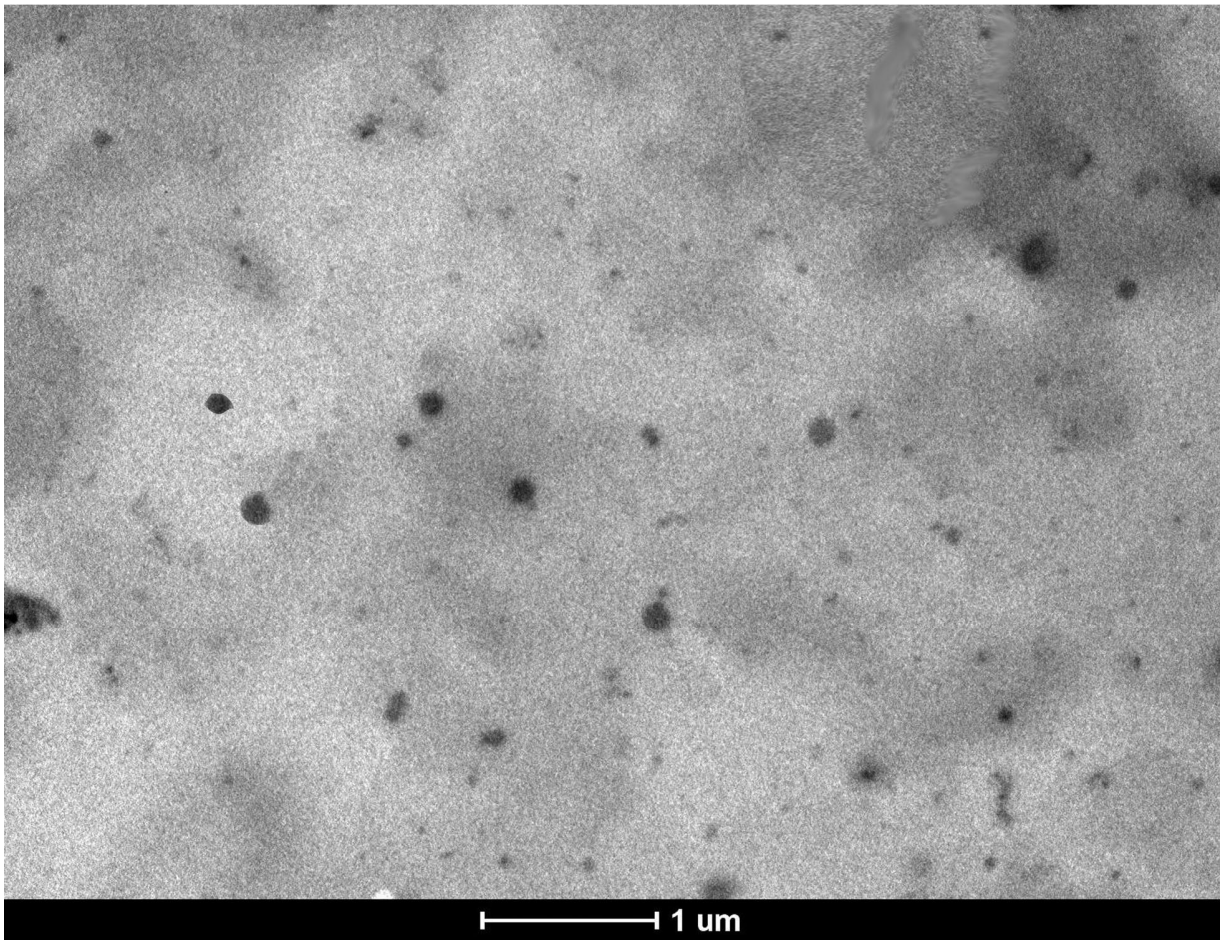



**Figure.15B TEM IMAGE OF NIOSOMAL GEL FORMULATIONS (FG2)**

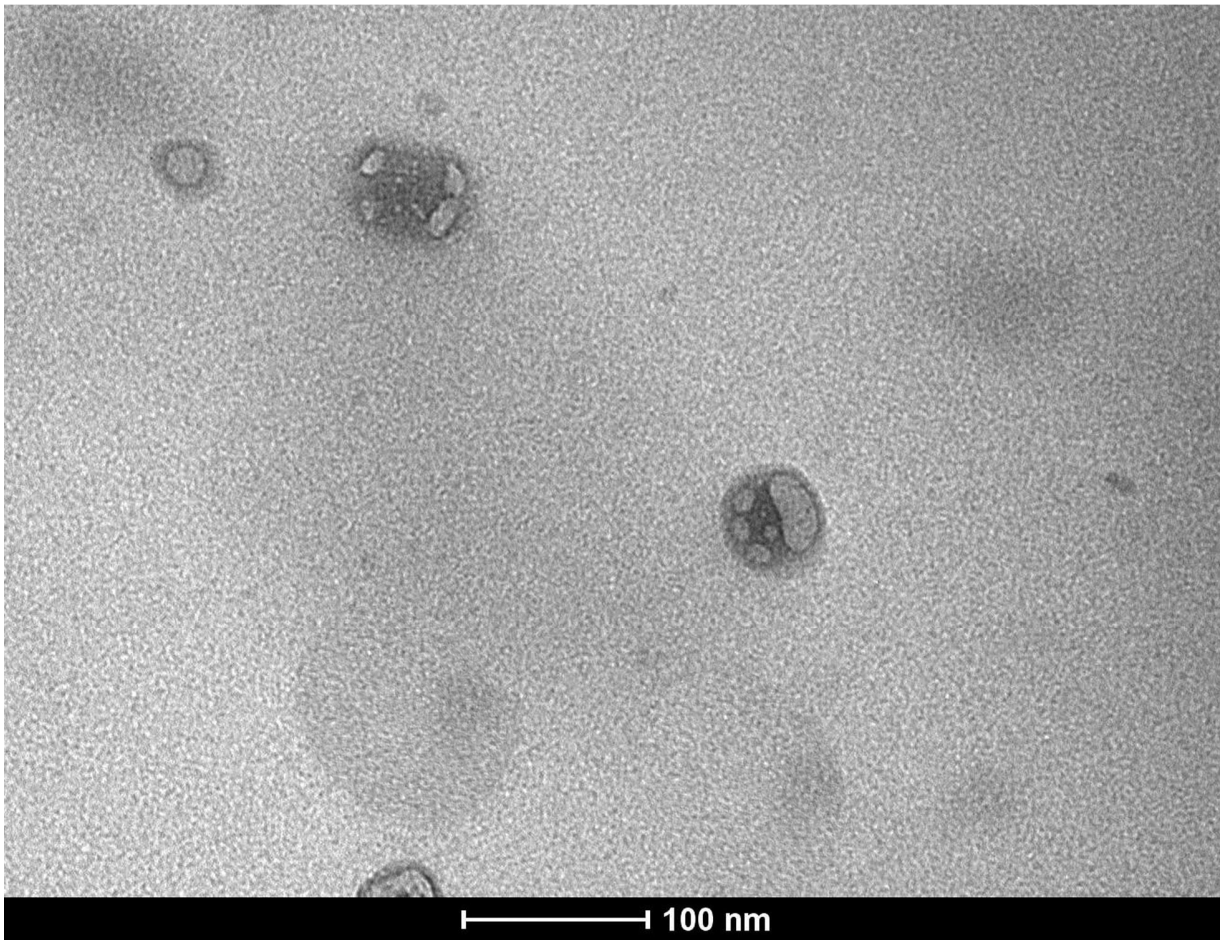



**Figure.15C TEM IMAGE OF NIOSOMAL GEL FORMULATIONS (FG3)**

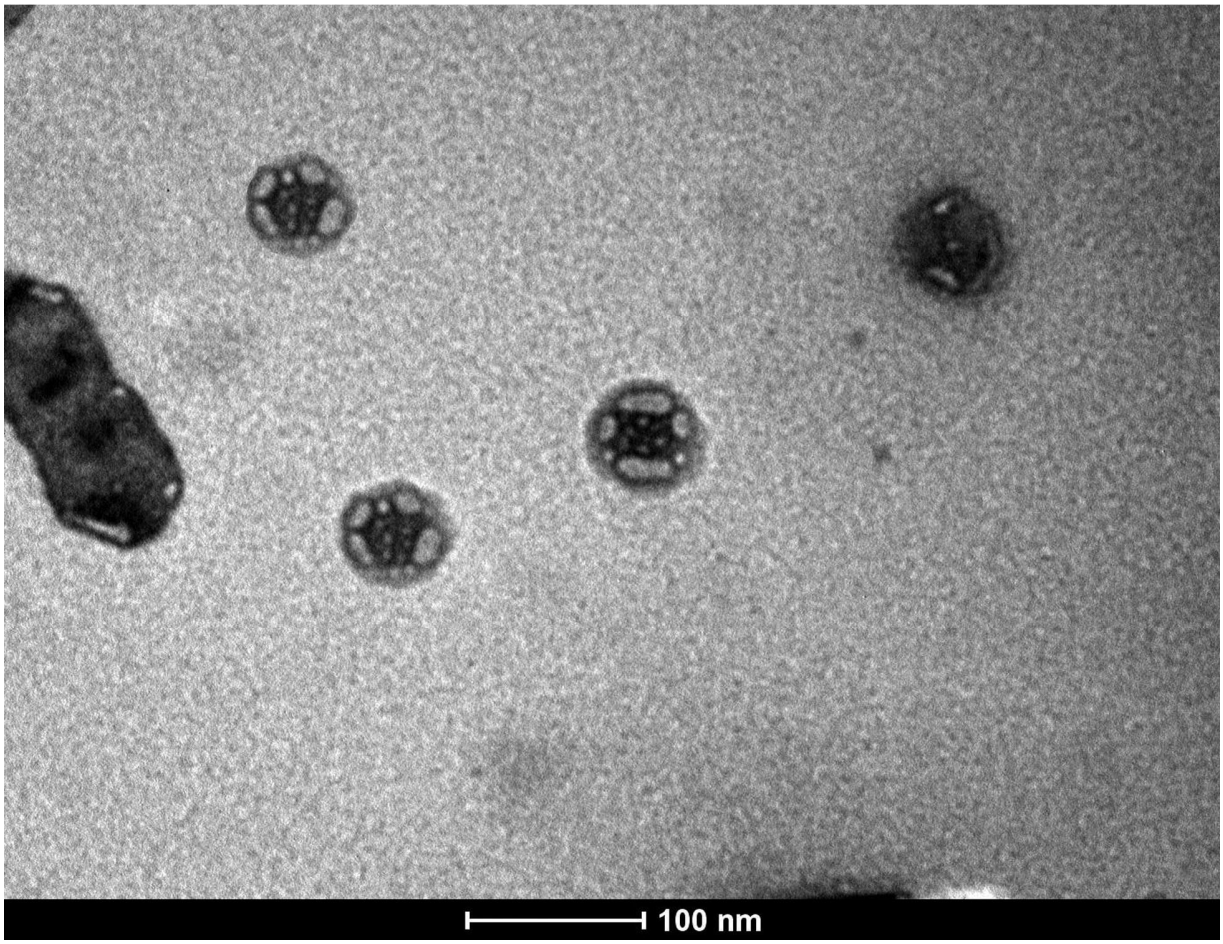



**Figure.15D TEM IMAGE OF NIOSOMAL GEL FORMULATIONS (FG3)**

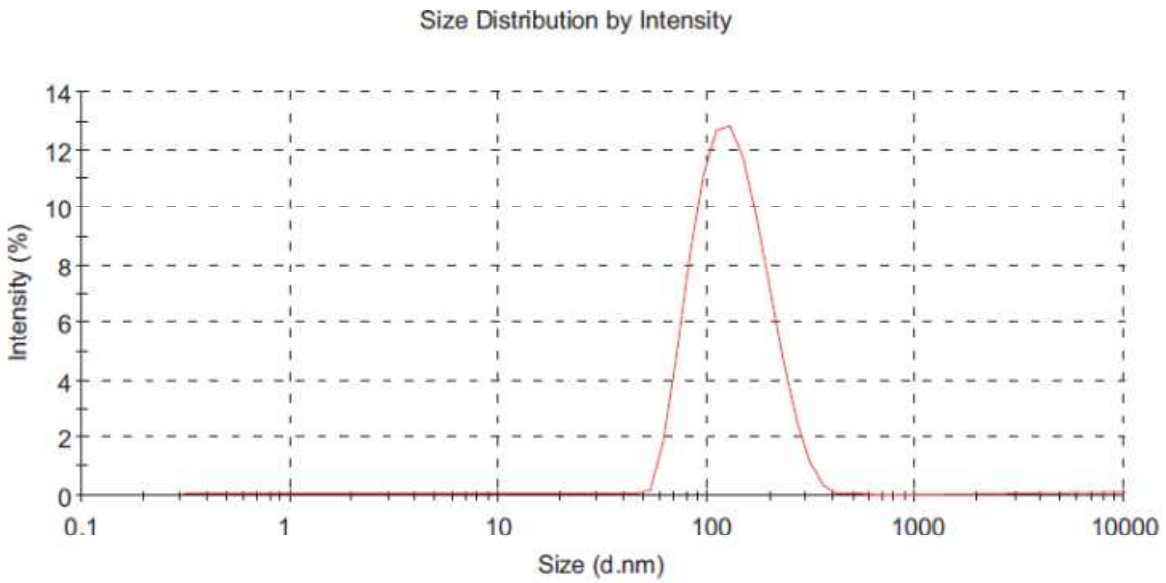



**Figure.16A PARTICLE SIZE DISTRIBUTION OF FG2**

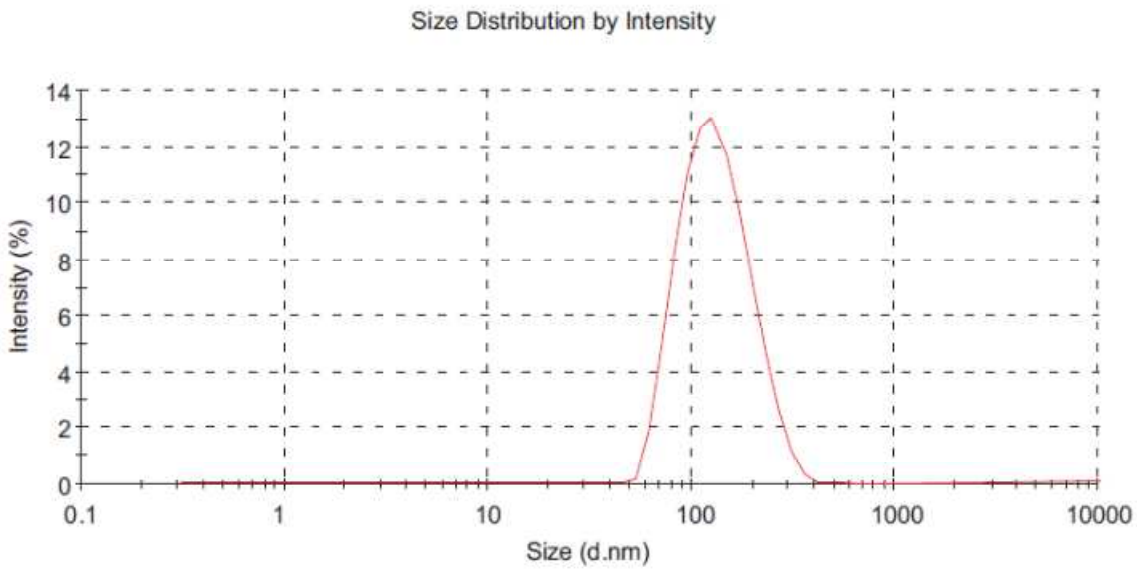



**Figure.16B PARTICLE SIZE DISTRIBUTION OF FG3**

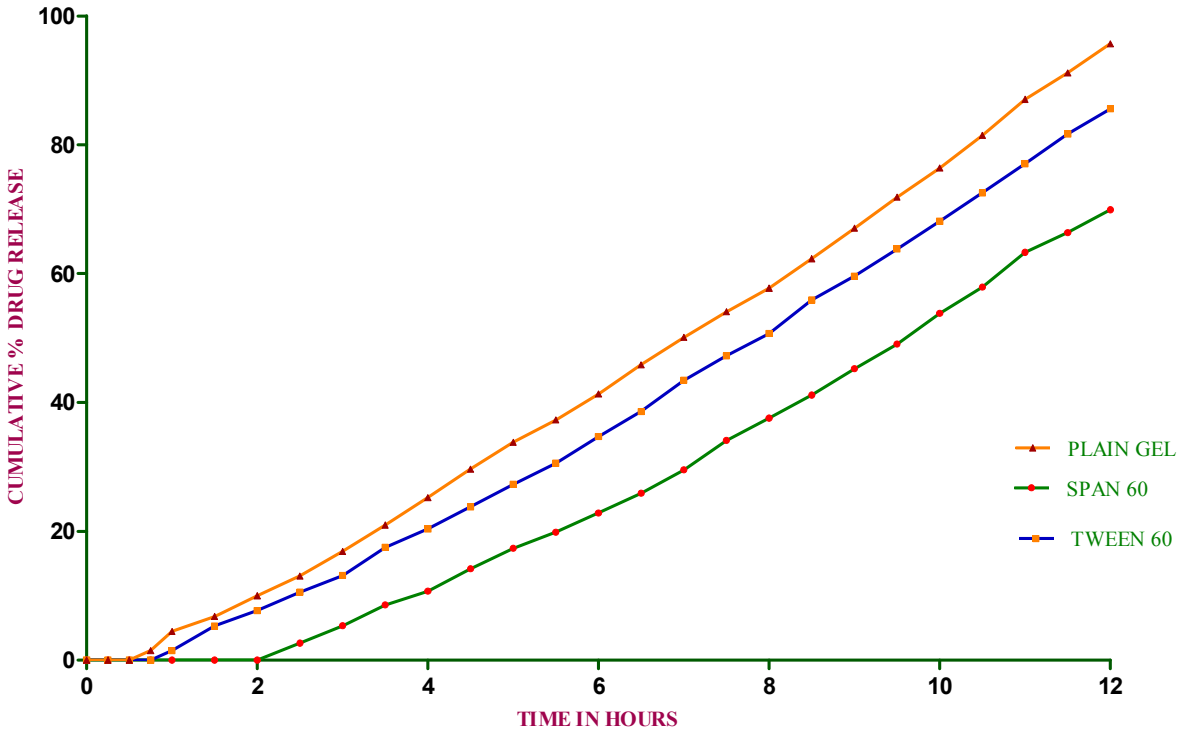
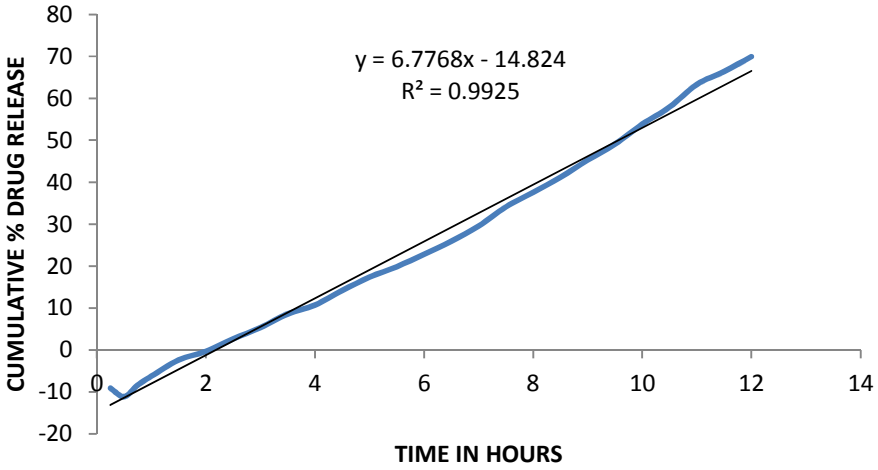
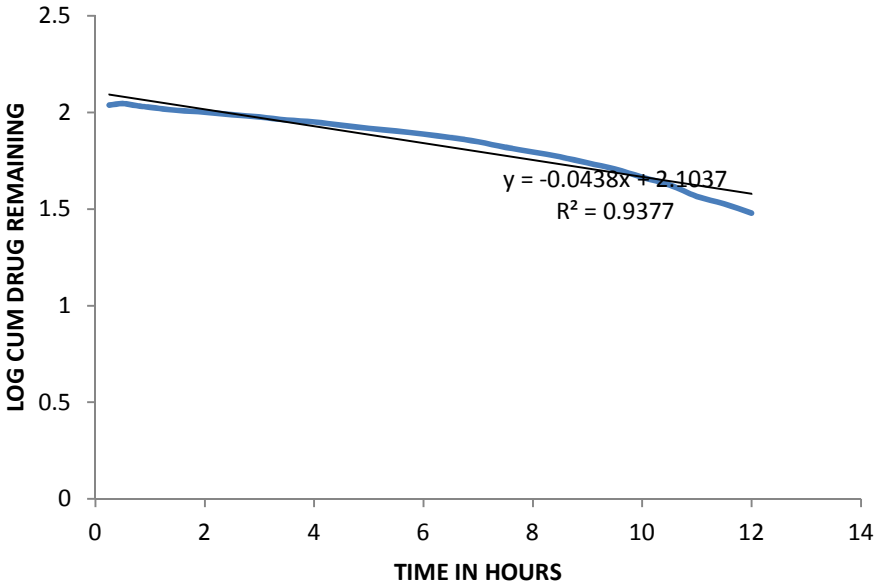



**Figure.17 COMPARISON OF INVITRO RELEASE OF LORNOXICAM PLAIN GEL – FG1 AND LORNOXICAM NIOSOMAL GEL FG2 AND FG3**

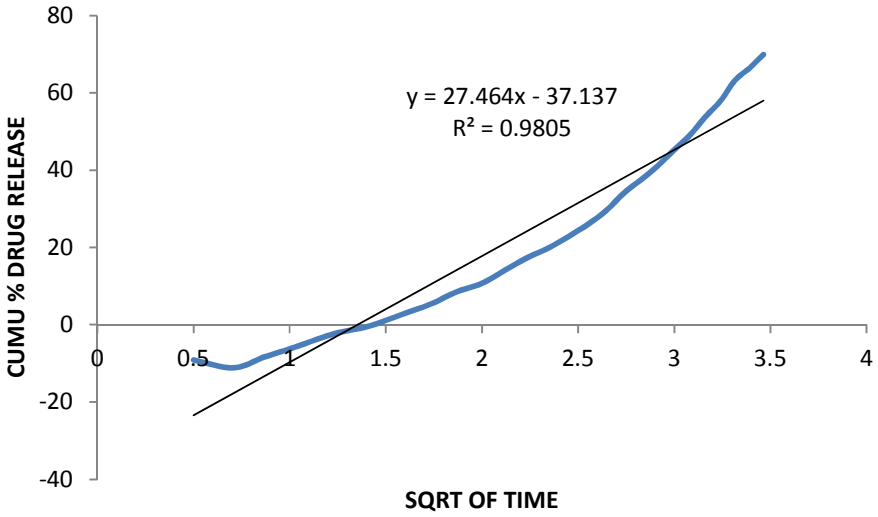
## A) ZERO ORDER



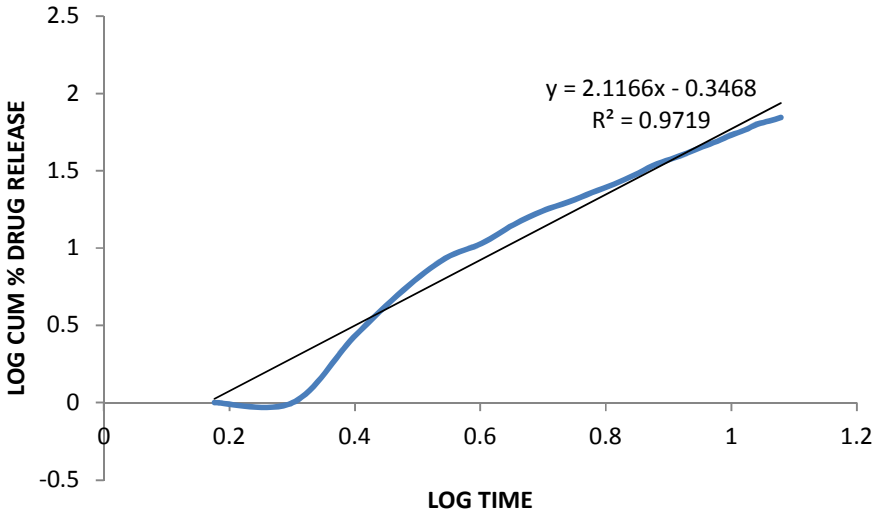

## B) FIRST ORDER



## C) HIGUCHI RELEASE KINETICS




## D) KORESEMEYER PEPPAS MODEL



## E) HIXON CROWELL

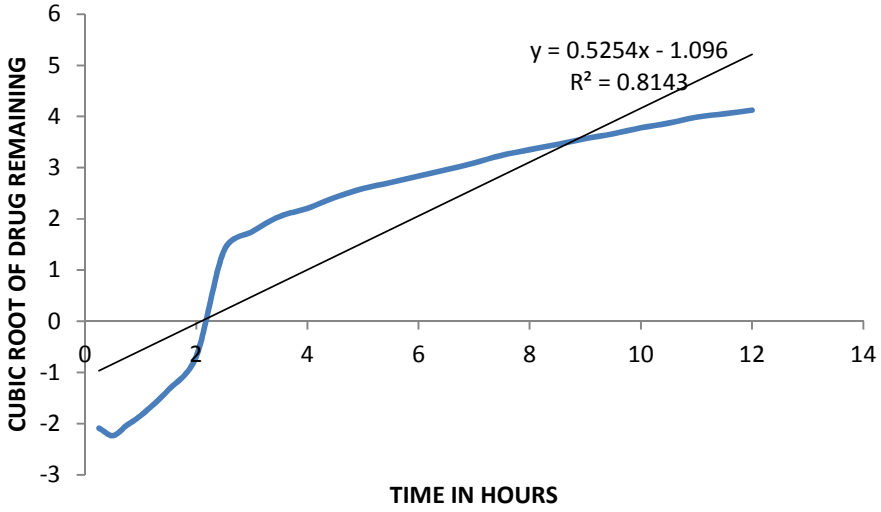



**Figure.18 COMPARISON OF IN VITRO RELEASE KINETICS OF SPAN 60 LORNOXICAM NIOSOMAL GEL (FG2)**

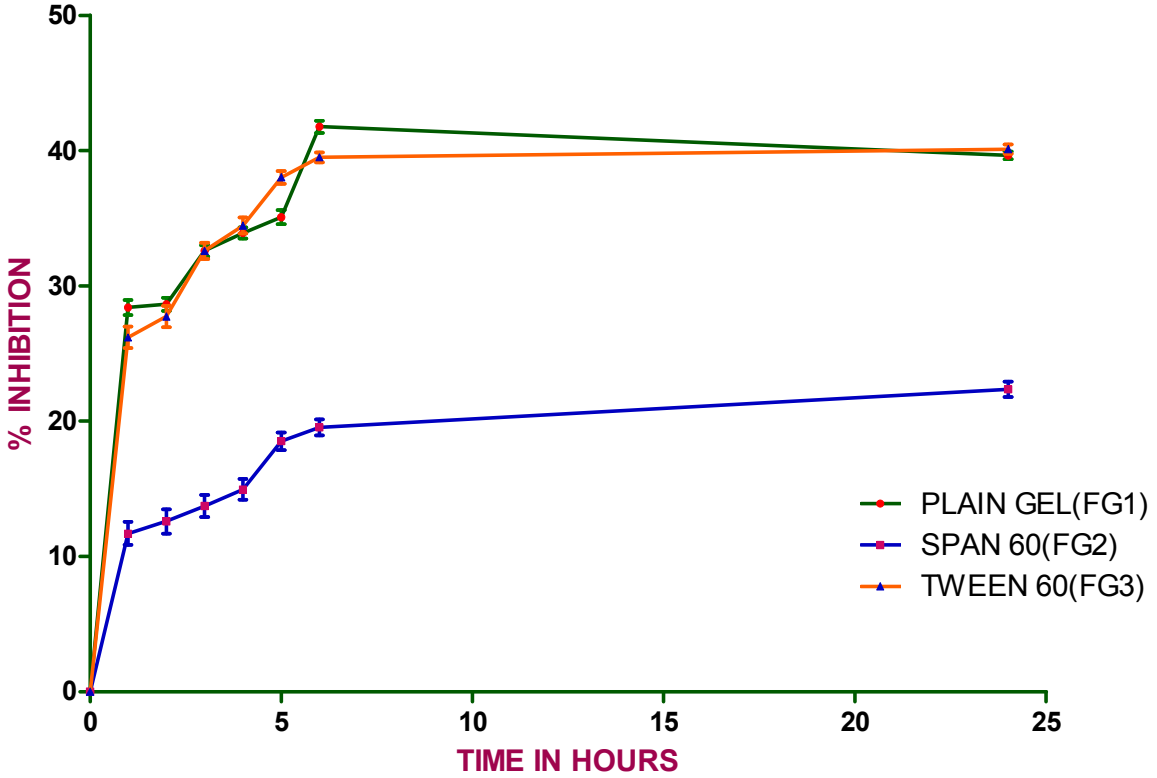



**Figure.19 COMPARISON OF PERCENTAGE INHIBITION OF RAT PAW EDEMA USING PLAIN LORNOXICAM GEL – FG1 AND LORNOXICAM NIOSOMALGEL FG2 AND FG3.**

**TABLE. 3 CALIBRATION CURVE OF LORNOXICAM IN PHOSPHATE BUFFERED SALINE pH 7.4**

| S.No. | CONCENTRATION (µg/ml) | ABSORBANCE ± SD* |
|---|---|---|
| 1. | 2 | 0.096 ± 0.019 |
| 2. | 4 | 0.187 ± 0.026 |
| 3. | 6 | 0.273 ± 0.016 |
| 4. | 8 | 0.341 ± 0.007 |
| 5. | 10 | 0.408 ± 0.003 |
| 6. | 12 | 0.494 ± 0.002 |
| 7. | 14 | 0.584 ± 0.007 |
| 8. | 16 | 0.659 ± 0.004 |
| 9. | 18 | 0.732 ± 0.026 |
| 10. | 20 | 0.843 ± 0.013 |

n = 3* **γ = 0.99908**

**TABLE. 4 FORMULATIONS OF LORNOXICAM NIOSOMES**

| S.NO | FORMULATION | SURFACTANT | RATIO OF | |
|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL |
| 1. | F1 | SPAN 40 | 4 | 1 |
| 2. | F2 | SPAN 40 | 6 | 1 |
| 3. | F3 | SPAN 40 | 8 | 1 |
| 4. | F4 | SPAN 40 | 10 | 1 |
| 5. | F5 | SPAN 60 | 4 | 1 |
| 6. | F6 | SPAN 60 | 6 | 1 |
| 7. | F7 | SPAN 60 | 8 | 1 |
| 8. | F8 | SPAN 60 | 10 | 1 |
| 9. | F9 | SPAN 80 | 4 | 1 |
| 10. | F10 | SPAN 80 | 6 | 1 |

Drug concentration used in each formulation kept as constant 20mg/10ml.

In ratio 1 stands for 25µmol.

**TABLE . 5 FORMULATION OF LORNOXICAM NIOSOMES**

| S.NO | FORMULATION | SURFACTANT | RATIO OF | |
|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL |
| 1. | F11 | SPAN 80 | 8 | 1 |
| 2. | F12 | SPAN 80 | 10 | 1 |
| 3. | F13 | TWEEN 20 | 4 | 1 |
| 4. | F14 | TWEEN 20 | 6 | 1 |
| 5. | F15 | TWEEN 20 | 8 | 1 |
| 6. | F16 | TWEEN 20 | 10 | 1 |
| 7. | F17 | TWEEN 40 | 4 | 1 |
| 8. | F18 | TWEEN 40 | 6 | 1 |
| 9. | F19 | TWEEN 40 | 8 | 1 |
| 10. | F20 | TWEEN 40 | 10 | 1 |
| 11. | F21 | TWEEN 60 | 4 | 1 |

Drug concentration used in each formulation kept as constant 20mg/10ml.

In ratio 1 stands for 25µmol.

**TABLE . 6 FORMULATION OF LORNOXICAM NIOSOMES**

| S.NO | FORMULATION | SURFACTANT | RATIO OF | |
|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL |
| 1. | F22 | TWEEN 60 | 6 | 1 |
| 2. | F23 | TWEEN 60 | 8 | 1 |
| 3. | F24 | TWEEN 60 | 10 | 1 |
| 4. | F25 | TWEEN 80 | 4 | 1 |
| 5. | F26 | TWEEN 80 | 6 | 1 |
| 6. | F27 | TWEEN 80 | 8 | 1 |
| 7. | F28 | TWEEN 80 | 10 | 1 |
| 8. | F29 | BRIJ 52 | 4 | 1 |
| 9. | F30 | BRIJ 52 | 6 | 1 |
| 10. | F31 | BRIJ 52 | 8 | 1 |
| 11. | F32 | BRIJ 52 | 10 | 1 |

Drug concentration used in each formulation kept as constant 20mg/10ml.

In ratio 1 stands for 25µmol.

**TABLE . 7 % ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS**

| S.NO | FORMULATION | SURFACTANT | RATIO | | % ENTRAPMENT |
|---|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL | |
| 1. | F1 | SPAN 40 | 4 | 1 | 75.86 |
| 2. | F2 | SPAN 40 | 6 | 1 | 76.38 |
| 3. | F3 | SPAN 40 | 8 | 1 | 77.13 |
| 4. | F4 | SPAN 40 | 10 | 1 | 79.26 |
| 5. | F5 | SPAN 60 | 4 | 1 | 84.38 |
| 6. | F6 | SPAN 60 | 6 | 1 | 86.69 |
| 7. | F7 | SPAN 60 | 8 | 1 | 87.06 |
| 8. | F8 | SPAN 60 | 10 | 1 | 89.91 |
| 9. | F9 | SPAN 80 | 4 | 1 | 66.75 |
| 10. | F10 | SPAN 80 | 6 | 1 | 68.48 |

**TABLE. 8 % ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS**

| S.NO | FORMULATION | SURFACTANT | RATIO | | % ENTRAPMENT |
|---|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL | |
| 1. | F11 | SPAN 80 | 8 | 1 | 69.51 |
| 2. | F12 | SPAN 80 | 10 | 1 | 70.27 |
| 3. | F13 | TWEEN 20 | 4 | 1 | 70.13 |
| 4. | F14 | TWEEN 20 | 6 | 1 | 71.14 |
| 5. | F15 | TWEEN 20 | 8 | 1 | 72.31 |
| 6. | F16 | TWEEN 20 | 10 | 1 | 73.77 |
| 7. | F17 | TWEEN 40 | 4 | 1 | 74.03 |
| 8. | F18 | TWEEN 40 | 6 | 1 | 75.37 |
| 9. | F19 | TWEEN 40 | 8 | 1 | 77.28 |
| 10. | F20 | TWEEN 40 | 10 | 1 | 79.23 |
| 11. | F21 | TWEEN 60 | 4 | 1 | 86.69 |

**TABLE . 9 % ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS**

| S.NO | FORMULATION | SURFACTANT | RATIO | | % ENTRAPMENT |
|---|---|---|---|---|---|
| | | | SURFACTANT | CHOLESTEROL | |
| 1. | F22 | TWEEN 60 | 6 | 1 | 87.44 |
| 2. | F23 | TWEEN 60 | 8 | 1 | 88.72 |
| 3. | F24 | TWEEN 60 | 10 | 1 | 89.48 |
| 4. | F25 | TWEEN 80 | 4 | 1 | 67.75 |
| 5. | F26 | TWEEN 80 | 6 | 1 | 69.51 |
| 6. | F27 | TWEEN 80 | 8 | 1 | 70.55 |
| 7. | F28 | TWEEN 80 | 10 | 1 | 71.24 |
| 8. | F29 | BRIJ 52 | 4 | 1 | 88.19 |
| 9. | F30 | BRIJ 52 | 6 | 1 | 89.21 |
| 10. | F31 | BRIJ 52 | 8 | 1 | 90.25 |
| 11. | F32 | BRIJ 52 | 10 | 1 | 92.05 |

**TABLE . 10 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | **F1 (SPAN 40 4:1)** | **F2 (SPAN 40 6:1)** | **F3 (SPAN 40 8:1)** | **F4 (SPAN 40 10:1)** |
| 0.25 | 7.73 ±0.49 | 5.06 ±0.33 | 3.20 ±0.49 | 1.60 ±0.24 |
| 0.5 | 9.30 ±1.32 | 7.05 ±0.34 | 5.23 ±0.52 | 3.29 ±0.23 |
| 0.75 | 12.00 ±0.54 | 9.39 ±0.35 | 7.60 ±0.79 | 4.88 ±0.16 |
| 1.0 | 16.56 ±0.57 | 11.76 ±0.29 | 10.03 ±0.57 | 6.84 ±0.17 |
| 1.5 | 18.32 ±0.59 | 14.30 ±0.31 | 12.50 ±0.59 | 9.33 ±0.18 |
| 2.0 | 20.92 ±0.62 | 16.47 ±0.41 | 14.70 ±0.61 | 12.02 ±0.19 |
| 2.5 | 23.22 ±0.64 | 18.65 ±0.34 | 17.30 ±0.81 | 14.83 ±0.28 |
| 3.0 | 25.99 ±0.67 | 21.44 ±0.35 | 21.10 ±0.66 | 18.41 ±0.29 |
| 3.5 | 31.04 ±0.69 | 24.06 ±0.45 | 23.80 ±0.63 | 21.25 ±0.31 |
| 4.0 | 32.97 ±0.71 | 27.03 ±0.47 | 26.50 ±0.73 | 24.16 ±0.23 |
| 4.5 | 35.66 ±0.24 | 29.84 ±0.47 | 29.00 ±0.84 | 25.63 ±0.32 |
| 5.0 | 37.88 ±0.33 | 32.73 ±0.41 | 31.90 ±0.80 | 28.42 ±0.34 |
| 5.5 | 40.55 ±0.30 | 35.71 ±0.51 | 34.30 ±0.82 | 30.27 ±0.26 |
| 6.0 | 43.02 ±0.33 | 38.78 ±0.51 | 36.90 ±0.92 | 37.10 ±0.36 |
| 6.5 | 46.54 ±0.33 | 41.93 ±0.46 | 40.50 ±0.86 | 39.80 ±0.29 |
| 7.0 | 51.50 ±0.37 | 45.17 ±0.56 | 43.20 ±0.90 | 42.30 ±0.29 |
| 7.5 | 56.61 ±0.37 | 48.76 ±0.57 | 46.00 ±0.92 | 46.80 ±0.02 |
| 8.0 | 59.29 ±0.42 | 52.66 ±0.59 | 48.80 ±1.02 | 48.90 ±0.03 |
| 8.5 | 63.50 ±0.42 | 57.46 ±0.61 | 52.40 ±0.98 | 51.70 ±0.03 |
| 9.0 | 68.31 ±0.51 | 61.41 ±0.63 | 57.00 ±1.00 | 54.95 ±0.04 |
| 9.5 | 73.08 ±0.47 | 65.20 ±0.63 | 58.70 ±1.33 | 57.39 ±0.05 |
| 10.0 | 78.17 ±0.52 | 70.61 ±0.66 | 62.20 ±1.13 | 60.11 ±0.06 |
| 10.5 | 83.67 ±0.54 | 74.64 ±0.66 | 67.50 ±1.07 | 63.83 ±0.07 |
| 11.0 | 89.45 ±0.72 | 79.03 ±0.68 | 71.80 ±0.39 | 66.65 ±0.16 |
| 11.5 | 91.84 ±0.66 | 83.19 ±0.71 | 75.30 ±1.13 | 69.65 ±0.17 |
| 12.0 | 95.63 ±0.51 | 87.56 ±0.72 | 79.30 ±1.15 | 72.70 ±0.19 |

n = 3*

**TABLE . 11 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F5 (SPAN 60 4:1) | F6 (SPAN 60 6:1) | F7 (SPAN 60 8:1) | F8 (SPAN 60 10:1) |
| 0.25 | 7.70 ±0.49 | 6.26 ±0.60 | 2.06 ±0.40 | 1.23 ±0.25 |
| 0.5 | 9.58 ±0.52 | 7.58 ±0.63 | 3.37 ±0.42 | 2.52 ±0.26 |
| 0.75 | 12.94 ±0.54 | 8.84 ±0.51 | 5.03 ±0.44 | 3.28 ±0.38 |
| 1.0 | 15.55 ±0.57 | 10.58 ±0.73 | 6.77 ±0.46 | 4.54 ±0.04 |
| 1.5 | 18.25 ±0.59 | 12.52 ±0.71 | 8.31 ±0.44 | 5.98 ±0.29 |
| 2.0 | 21.32 ±0.57 | 14.57 ±0.74 | 10.69 ±0.45 | 7.75 ±0.30 |
| 2.5 | 23.97 ±0.64 | 16.69 ±0.77 | 12.36 ±0.47 | 9.60 ±0.31 |
| 3.0 | 26.48 ±0.67 | 18.63 ±0.86 | 14.40 ±0.19 | 11.52 ±0.33 |
| 3.5 | 29.26 ±0.69 | 20.65 ±0.84 | 16.01 ±0.49 | 13.22 ±0.34 |
| 4.0 | 31.82 ±0.87 | 21.98 ±0.87 | 18.17 ±0.51 | 15.27 ±0.35 |
| 4.5 | 34.66 ±0.75 | 24.79 ±0.90 | 19.67 ±0.57 | 16.90 ±0.36 |
| 5.0 | 36.61 ±0.88 | 26.70 ±0.98 | 21.44 ±0.59 | 18.32 ±0.38 |
| 5.5 | 39.32 ±0.80 | 28.20 ±1.01 | 23.27 ±0.61 | 20.30 ±0.39 |
| 6.0 | 42.44 ±0.83 | 30.49 ±0.99 | 25.11 ±0.58 | 22.59 ±0.40 |
| 6.5 | 45.74 ±0.85 | 32.24 ±1.53 | 28.26 ±0.65 | 25.88 ±0.56 |
| 7.0 | 48.63 ±0.90 | 35.69 ±1.08 | 31.29 ±0.67 | 27.69 ±0.23 |
| 7.5 | 51.68 ±0.90 | 37.99 ±0.90 | 34.43 ±0.69 | 29.87 ±0.60 |
| 8.0 | 54.41 ±0.93 | 41.14 ±1.18 | 37.59 ±0.66 | 32.62 ±0.75 |
| 8.5 | 57.18 ±0.95 | 44.29 ±1.16 | 40.42 ±0.68 | 35.71 ±0.90 |
| 9.0 | 60.21 ±0.98 | 46.99 ±1.24 | 43.33 ±0.70 | 38.77 ±0.78 |
| 9.5 | 63.39 ±1.00 | 49.78 ±1.22 | 45.58 ±0.76 | 41.52 ±0.59 |
| 10.0 | 66.34 ±1.03 | 53.11 ±1.30 | 48.83 ±0.74 | 44.44 ±0.83 |
| 10.5 | 69.04 ±1.05 | 57.49 ±1.34 | 53.40 ±0.80 | 47.33 ±0.81 |
| 11.0 | 71.88 ±1.08 | 62.07 ±1.32 | 57.32 ±0.81 | 50.73 ±1.12 |
| 11.5 | 74.65 ±1.10 | 65.76 ±1.40 | 60.35 ±0.93 | 54.02 ±0.85 |
| 12.0 | 77.46 ±1.13 | 71.27 ±1.38 | 63.27 ±0.95 | 57.19 ±0.81 |

n = 3*

**TABLE. 12 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F9 (SPAN 80 4:1) | F10 (SPAN 80 6:1) | F11 (SPAN 80 8:1) | F12 (SPAN 80 10:1) |
| 0.25 | 6.20 ±0.32 | 5.20 ±0.48 | 4.60 ±0.66 | 3.80 ±0.24 |
| 0.5 | 8.20±0.35 | 9.50 ±0.52 | 6.30 ±0.25 | 5.50 ±0.17 |
| 0.75 | 10.40±0.37 | 13.10 ±0.54 | 8.10 ±0.18 | 7.30 ±0.26 |
| 1.0 | 12.80±0.29 | 16.50 ±0.57 | 10.30 ±0.19 | 9.40 ±0.19 |
| 1.5 | 15.40 ±0.31 | 19.80 ±0.59 | 11.40 ±0.55 | 11.60 ±0.20 |
| 2.0 | 18.30 ±0.41 | 23.20 ±1.97 | 15.90 ±0.29 | 13.80 ±0.37 |
| 2.5 | 21.10 ±0.43 | 26.50 ±1.54 | 18.30 ±0.20 | 16.98 ±0.22 |
| 3.0 | 24.00 ±0.35 | 30.50 ±2.35 | 22.10 ±0.24 | 19.60 ±0.23 |
| 3.5 | 27.70 ±0.44 | 33.40 ±1.68 | 24.60 ±0.34 | 22.90 ±0.24 |
| 4.0 | 34.00 ±0.46 | 36.70 ±1.59 | 27.30 ±0.26 | 26.14 ±0.33 |
| 4.5 | 36.70 ±0.40 | 40.40 ±2.26 | 29.70 ±0.36 | 28.40 ±0.34 |
| 5.0 | 39.90 ±0.49 | 43.30 ±1.83 | 31.60 ±0.29 | 31.08 ±0.29 |
| 5.5 | 43.40 ±0.50 | 45.80 ±1.67 | 34.60 ±0.29 | 33.70 ±0.36 |
| 6.0 | 46.50 ±0.44 | 49.20 ±1.54 | 37.80 ±0.17 | 36.50 ±0.29 |
| 6.5 | 48.90 ±0.53 | 51.40 ±1.16 | 43.10 ±0.25 | 39.80 ±0.30 |
| 7.0 | 52.20 ±0.56 | 54.20 ±0.85 | 46.10 ±0.68 | 42.80 ±0.39 |
| 7.5 | 56.10 ±0.58 | 56.80 ±0.87 | 50.70 ±0.30 | 45.50 ±0.40 |
| 8.0 | 59.70 ±0.50 | 59.60 ±0.93 | 54.30 ±0.32 | 48.60 ±0.33 |
| 8.5 | 63.90 ±0.52 | 62.40 ±0.72 | 57.70 ±0.26 | 51.70 ±0.42 |
| 9.0 | 69.50 ±0.62 | 65.70 ±1.07 | 61.40 ±0.35 | 55.50 ±0.44 |
| 9.5 | 73.80 ±0.64 | 70.40 ±0.91 | 66.50 ±0.36 | 58.80 ±0.36 |
| 10.0 | 78.20 ±0.65 | 76.10 ±0.70 | 70.10 ±0.30 | 61.90 ±0.41 |
| 10.5 | 82.70 ±0.70 | 82.90 ±0.51 | 74.40 ±0.31 | 64.90 ±0.38 |
| 11.0 | 86.50 ±0.69 | 84.56 ±0.51 | 78.10 ±0.41 | 67.90 ±0.39 |
| 11.5 | 90.40 ±0.69 | 86.40 ±0.52 | 82.20 ±0.34 | 71.50 ±0.48 |
| 12.0 | 94.40 ±0.63 | 90.90 ±0.49 | 85.70 ±0.43 | 74.30 ±0.41 |

n = 3*

**TABLE. 13 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F13 (TWEEN 20 4:1) | F14 (TWEEN 20 6:1) | F15 (TWEEN 20 8:1) | F16 (TWEEN 20 10:1) |
| 0.25 | 4.60 ±0.57 | 3.70 ±0.33 | 2.20 ±0.16 | 1.20 ±0.16 |
| 0.5 | 6.50 ±0.44 | 5.30 ±0.34 | 4.20 ±0.25 | 2.40 ±0.19 |
| 0.75 | 8.40 ±0.70 | 7.10 ±0.37 | 6.40 ±0.26 | 4.30 ±0.16 |
| 1.0 | 10.50 ±0.83 | 9.20 ±0.29 | 8.40 ±0.31 | 5.70 ±0.17 |
| 1.5 | 13.00 ±0.61 | 10.10 ±0.40 | 15.50 ±0.22 | 7.50 ±0.27 |
| 2.0 | 16.90 ±1.73 | 14.70 ±0.41 | 12.70 ±0.30 | 9.41 ±0.20 |
| 2.5 | 21.70 ±2.21 | 17.40 ±0.44 | 15.30 ±0.37 | 11.00 ±0.29 |
| 3.0 | 24.50 ±2.11 | 21.00 ±0.45 | 17.90 ±0.32 | 12.90 ±0.30 |
| 3.5 | 28.10 ±2.50 | 24.40 ±0.41 | 20.70 ±0.33 | 15.00 ±0.23 |
| 4.0 | 31.30 ±2.39 | 26.90 ±0.18 | 23.50 ±0.37 | 17.40 ±0.32 |
| 4.5 | 34.90 ±2.03 | 28.90 ±0.13 | 26.20 ±0.29 | 19.40 ±0.25 |
| 5.0 | 39.80 ±2.83 | 31.50 ±0.45 | 29.50 ±0.39 | 21.60 ±0.34 |
| 5.5 | 43.61 ±2.53 | 34.70 ±0.75 | 32.70 ±1.44 | 24.90 ±0.43 |
| 6.0 | 47.93 ±2.04 | 39.90 ±1.95 | 37.70 ±0.49 | 26.70 ±0.21 |
| 6.5 | 51.00 ±1.73 | 45.50 ±0.88 | 40.90 ±0.41 | 28.90 ±0.47 |
| 7.0 | 55.20 ±1.84 | 48.90 ±1.28 | 44.70 ±0.36 | 31.70 ±0.68 |
| 7.5 | 59.80 ±1.99 | 52.60 ±0.45 | 48.30 ±0.43 | 34.80 ±0.80 |
| 8.0 | 63.50 ±2.07 | 55.80 ±0.56 | 52.00 ±0.44 | 37.80 ±0.66 |
| 8.5 | 67.60 ±1.81 | 59.50 ±0.67 | 56.10 ±0.39 | 40.60 ±0.50 |
| 9.0 | 71.80 ±0.98 | 64.20 ±1.26 | 60.30 ±0.40 | 43.30 ±0.65 |
| 9.5 | 75.50 ±1.28 | 68.20 ±0.66 | 64.60 ±0.36 | 46.30 ±0.72 |
| 10.0 | 79.20 ±1.30 | 72.40 ±0.87 | 68.50 ±0.40 | 49.80 ±0.92 |
| 10.5 | 83.20 ±1.50 | 76.30 ±0.67 | 72.10 ±0.42 | 53.10 ±0.71 |
| 11.0 | 87.70 ±1.56 | 80.20 ±0.82 | 76.10 ±0.43 | 56.20 ±0.74 |
| 11.5 | 92.80 ±1.36 | 83.70 ±0.35 | 79.60 ±0.41 | 59.10 ±0.41 |
| 12.0 | 97.80 ±1.25 | 86.90 ±0.20 | 80.00 ±0.43 | 61.50 ±0.99 |

n = 3*

**TABLE . 14 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F17 (TWEEN 40 4:1) | F18 (TWEEN 40 6:1) | F19 (TWEEN 40 8:1) | F20 (TWEEN 40 10:1) |
| 0.25 | 5.93±0.33 | 3.26±0.33 | 1.73±0.49 | 0.86±0.33 |
| 0.5 | 8.23±0.35 | 4.96±0.34 | 3.83±0.45 | 2.57±0.26 |
| 0.75 | 10.16±0.28 | 6.67±0.37 | 5.96±0.54 | 3.77±0.14 |
| 1.0 | 14.56±0.37 | 8.78±0.38 | 7.96±0.64 | 6.61±0.36 |
| 1.5 | 16.23±0.38 | 10.38±0.40 | 10.10±0.58 | 8.93±0.38 |
| 2.0 | 18.69±0.41 | 14.31±0.33 | 12.26±0.62 | 11.34±0.39 |
| 2.5 | 27.03±0.34 | 16.95±0.34 | 14.83±0.64 | 13.85±0.41 |
| 3.0 | 23.28±0.68 | 20.42±0.44 | 17.50±0.67 | 16.20±0.41 |
| 3.5 | 28.25±0.69 | 22.64±0.53 | 20.33±0.69 | 18.63±0.35 |
| 4.0 | 30.22±0.71 | 25.32±0.63 | 23.06±0.72 | 23.89±0.44 |
| 4.5 | 34.92±1.13 | 27.62±0.56 | 25.66±0.73 | 26.57±0.90 |
| 5.0 | 39.02±1.05 | 29.53±0.56 | 29.05±0.84 | 30.74±0.40 |
| 5.5 | 41.79±1.16 | 32.46±0.58 | 33.06±0.78 | 33.50±0.67 |
| 6.0 | 44.36±1.17 | 35.88±0.60 | 37.36±0.81 | 37.53±0.43 |
| 6.5 | 47.91±1.10 | 41.75±0.50 | 40.32±0.84 | 39.72±0.53 |
| 7.0 | 53.09±1.20 | 45.51±0.58 | 45.16±1.66 | 42.01±0.55 |
| 7.5 | 58.24±1.16 | 50.38±0.60 | 47.75±0.89 | 44.68±0.50 |
| 8.0 | 61.03±1.23 | 53.24±0.62 | 51.26±1.07 | 46.87±0.56 |
| 8.5 | 65.31±1.24 | 56.84±0.56 | 55.43±0.95 | 49.49±0.58 |
| 9.0 | 70.31±1.26 | 60.84±0.67 | 59.69±0.96 | 52.09±0.52 |
| 9.5 | 75.11±1.22 | 65.58±0.70 | 63.87±0.99 | 54.81±0.53 |
| 10.0 | 80.31±1.29 | 70.01±0.49 | 67.73±1.02 | 57.11±0.63 |
| 10.5 | 85.80±1.36 | 73.54±0.62 | 71.29±1.04 | 59.90±0.65 |
| 11.0 | 89.85±1.32 | 77.18±0.70 | 75.30±1.07 | 62.75±0.67 |
| 11.5 | 94.19±1.24 | 81.35±0.65 | 78.73±1.10 | 65.34±0.67 |
| 12.0 | 98.00±1.26 | 84.80±0.75 | 82.15±1.12 | 67.40±0.70 |

n = 3*

**TABLE. 15 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F21 (TWEEN 60 4:1) | F22 (TWEEN 60 6:1) | F23 (TWEEN 60 8:1) | F24 (TWEEN 60 10:1) |
| 0.25 | 8.00 ±0.52 | 4.60 ±0.52 | 1.93 ±0.64 | 1.06 ±0.41 |
| 0.5 | 10.13 ±0.58 | 6.20 ±0.52 | 3.46 ±0.58 | 2.06 ±0.41 |
| 0.75 | 11.63 ±0.25 | 8.06 ±0.56 | 5.96 ±0.55 | 4.16 ±0.41 |
| 1.0 | 14.60 ±0.62 | 10.13 ±0.61 | 8.36 ±0.40 | 6.36 ±0.51 |
| 1.5 | 16.83 ±0.58 | 11.10 ±0.62 | 10.13 ±0.47 | 8.40 ±0.50 |
| 2.0 | 19.63 ±1.10 | 14.46 ±2.77 | 12.03 ±0.56 | 10.53 ±0.40 |
| 2.5 | 23.60 ±0.70 | 18.60 ±0.79 | 15.26 ±0.68 | 12.80 ±0.44 |
| 3.0 | 25.60 ±0.70 | 22.03 ±0.51 | 17.96 ±0.68 | 15.33 ±0.56 |
| 3.5 | 28.06 ±0.73 | 24.43 ±0.68 | 21.93 ±0.73 | 17.96 ±0.58 |
| 4.0 | 30.16 ±0.73 | 27.23 ±0.68 | 24.86 ±0.77 | 20.73 ±0.56 |
| 4.5 | 32.56 ±0.76 | 29.56 ±0.68 | 27.90 ±0.79 | 23.53 ±0.60 |
| 5.0 | 35.40 ±0.79 | 31.50 ±0.73 | 31.03 ±0.83 | 26.26 ±0.51 |
| 5.5 | 38.06 ±0.83 | 34.36 ±0.79 | 34.00 ±0.81 | 29.73 ±0.90 |
| 6.0 | 41.43 ±0.85 | 37.60 ±0.76 | 37.30 ±0.81 | 32.70 ±1.57 |
| 6.5 | 44.20 ±0.81 | 43.23 ±0.45 | 40.80 ±0.85 | 37.86 ±0.55 |
| 7.0 | 49.03 ±0.77 | 46.66 ±0.80 | 45.50 ±0.88 | 40.90 ±0.65 |
| 7.5 | 52.20 ±0.88 | 51.00 ±0.80 | 48.80 ±0.88 | 44.63 ±0.70 |
| 8.0 | 55.53 ±1.13 | 54.53 ±0.88 | 52.16 ±0.94 | 47.26 ±0.70 |
| 8.5 | 58.63 ±0.94 | 57.96 ±0.90 | 55.40 ±0.98 | 49.96 ±0.70 |
| 9.0 | 62.03 ±1.11 | 61.73 ±0.92 | 59.43 ±0.97 | 53.70 ±0.75 |
| 9.5 | 65.20 ±1.01 | 66.66 ±0.94 | 63.16 ±1.04 | 57.63 ±0.64 |
| 10.0 | 68.20 ±2.74 | 70.43 ±0.96 | 66.33 ±0.98 | 62.06 ±0.90 |
| 10.5 | 75.20 ±1.31 | 74.73 ±1.04 | 70.43 ±1.09 | 66.36 ±0.75 |
| 11.0 | 80.03 ±1.04 | 78.33 ±1.04 | 73.76 ±0.60 | 70.13 ±0.86 |
| 11.5 | 84.40 ±1.15 | 82.56 ±1.04 | 77.90 ±1.24 | 73.23 ±0.86 |
| 12.0 | 90.20 ±1.17 | 86.10 ±1.15 | 81.40 ±1.15 | 76.00 ±0.75 |

n = 3*

**TABLE. 16 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F25 (TWEEN 80 4:1) | F26 (TWEEN 80 6:1) | F27 (TWEEN 80 8:1) | F28 (TWEEN 80 10:1) |
| 0.25 | 4.10 ±0.65 | 3.08 ±0.37 | 1.58 ±0.37 | 1.42 ±0.37 |
| 0.5 | 5.53 ±0.63 | 4.72 ±0.39 | 3.65 ±0.39 | 3.15 ±0.53 |
| 0.75 | 7.30 ±0.66 | 6.41 ±0.37 | 5.07 ±0.41 | 4.79 ±0.32 |
| 1.0 | 9.64 ±0.69 | 8.31 ±0.55 | 6.06 ±0.43 | 6.84 ±0.43 |
| 1.5 | 11.78 ±0.72 | 9.35 ±0.44 | 8.08 ±0.45 | 9.40 ±0.45 |
| 2.0 | 14.58 ±0.80 | 13.95 ±0.49 | 10.18 ±0.47 | 12.02 ±0.42 |
| 2.5 | 18.22 ±0.84 | 16.57 ±0.51 | 12.29 ±0.47 | 14.77 ±0.51 |
| 3.0 | 21.74 ±0.82 | 20.02 ±0.49 | 14.99 ±0.37 | 18.43 ±0.53 |
| 3.5 | 25.13 ±0.90 | 24.08 ±0.51 | 17.47 ±0.50 | 21.23 ±0.55 |
| 4.0 | 28.18 ±0.93 | 26.37 ±0.57 | 20.20 ±0.52 | 24.10 ±0.53 |
| 4.5 | 31.57 ±0.91 | 28.19 ±0.55 | 23.04 ±0.54 | 25.60 ±0.55 |
| 5.0 | 34.53 ±0.94 | 31.07 ±0.61 | 25.67 ±0.55 | 28.35 ±0.61 |
| 5.5 | 38.14 ±1.03 | 34.51 ±0.59 | 28.96 ±0.61 | 30.23 ±0.59 |
| 6.0 | 40.77 ±0.50 | 40.63 ±0.77 | 33.09 ±0.64 | 37.08 ±0.60 |
| 6.5 | 44.62 ±1.06 | 44.09 ±0.63 | 37.31 ±0.61 | 39.68 ±0.77 |
| 7.0 | 48.19 ±1.04 | 48.78 ±0.80 | 40.24 ±0.63 | 42.24 ±0.69 |
| 7.5 | 51.92 ±1.07 | 52.02 ±0.72 | 44.01 ±0.69 | 46.79 ±0.71 |
| 8.0 | 56.02 ±1.16 | 55.40 ±0.74 | 47.60 ±0.66 | 48.95 ±0.69 |
| 8.5 | 59.96 ±1.19 | 59.09 ±0.71 | 51.28 ±0.69 | 51.67 ±0.71 |
| 9.0 | 63.94 ±1.17 | 64.09 ±0.77 | 55.36 ±0.75 | 54.44 ±0.72 |
| 9.5 | 67.58 ±1.20 | 67.78 ±0.79 | 59.52 ±0.72 | 57.26 ±0.74 |
| 10.0 | 72.33 ±0.99 | 72.05 ±0.81 | 63.76 ±0.74 | 60.13 ±0.776 |
| 10.5 | 77.51 ±1.07 | 75.64 ±0.79 | 67.68 ±0.80 | 63.79 ±0.82 |
| 11.0 | 81.80 ±1.22 | 79.80 ±0.80 | 71.42 ±0.82 | 66.56 ±0.80 |
| 11.5 | 85.54 ±1.17 | 82.78 ±0.83 | 74.46 ±0.80 | 69.61 ±0.82 |
| 12.0 | 89.50 ±1.01 | 85.81 ±0.85 | 77.58 ±0.86 | 72.33 ±0.91 |

n = 3*

**TABLE . 17 COMPARISON OF INVITRO RELEASE PROFILE OF LORNOXICAM NIOSOMES**

| TIME IN HOURS | CUMULATIVE % DRUG RELEASE ± SD* | | | |
|---|---|---|---|---|
| | F29 (BRIJ 52 4:1) | F30 (BRIJ 52 6:1) | F31 (BRIJ 52 8:1) | F32 (BRIJ 52 10:1) |
| 0.25 | 6.90 ±0.50 | 5.76 ±0.40 | 4.60 ±0.36 | 2.53 ±0.35 |
| 0.5 | 8.74 ±0.52 | 7.35 ±0.28 | 6.96 ±0.30 | 4.29 ±0.26 |
| 0.75 | 10.66 ±0.55 | 8.64 ±0.18 | 9.40 ±0.43 | 5.60 ±0.43 |
| 1.0 | 13.16 ±0.57 | 9.80 ±0.13 | 10.50 ±0.19 | 6.86 ±0.43 |
| 1.5 | 14.95 ±0.60 | 11.14 ±0.26 | 12.05 ±0.31 | 8.57 ±0.29 |
| 2.0 | 17.11 ±0.62 | 12.83 ±0.24 | 13.21 ±0.21 | 9.96 ±0.31 |
| 2.5 | 19.64 ±0.65 | 14.54 ±0.43 | 15.70 ±0.20 | 11.56 ±0.17 |
| 3.0 | 22.16 ±0.67 | 16.24 ±0.30 | 18.42 ±0.12 | 14.08 ±0.17 |
| 3.5 | 27.06 ±0.70 | 18.29 ±0.46 | 21.28 ±0.13 | 16.11 ±0.29 |
| 4.0 | 30.03 ±0.89 | 20.62 ±0.33 | 24.02 ±0.15 | 17.61 ±0.21 |
| 4.5 | 34.06 ±0.68 | 22.93 ±0.35 | 27.97 ±0.15 | 19.05 ±0.19 |
| 5.0 | 38.10 ±0.71 | 25.84 ±0.22 | 30.65 ±0.16 | 21.46 ±0.37 |
| 5.5 | 41.38 ±0.73 | 28.76 ±0.41 | 34.80 ±0.41 | 25.48 ±0.39 |
| 6.0 | 44.94 ±0.76 | 31.01 ±0.41 | 38.47 ±0.32 | 28.84 ±0.16 |
| 6.5 | 47.81 ±0.78 | 33.44 ±0.39 | 41.09 ±0.11 | 32.34 ±0.36 |
| 7.0 | 51.73 ±0.81 | 36.21 ±0.72 | 43.33 ±0.07 | 35.53 ±0.27 |
| 7.5 | 54.87 ±0.83 | 40.90 ±0.43 | 46.15 ±0.20 | 38.99 ±0.17 |
| 8.0 | 58.77 ±0.86 | 44.35 ±0.44 | 48.82 ±0.21 | 42.35 ±0.24 |
| 8.5 | 63.47 ±0.62 | 48.23 ±0.64 | 51.95 ±0.21 | 44.78 ±0.43 |
| 9.0 | 67.50 ±0.42 | 51.93 ±0.52 | 55.19 ±0.27 | 47.85 ±0.16 |
| 9.5 | 70.97 ±0.43 | 55.94 ±0.57 | 58.73 ±0.22 | 51.00 ±0.08 |
| 10.0 | 74.46 ±0.46 | 62.15 ±0.49 | 62.73 ±0.42 | 54.75 ±0.29 |
| 10.5 | 77.71 ±0.55 | 65.95 ±0.45 | 66.30 ±0.85 | 58.81 ±0.55 |
| 11.0 | 80.84 ±0.53 | 68.90 ±0.51 | 69.35 ±0.16 | 62.28 ±0.33 |
| 11.5 | 83.91 ±0.72 | 73.06 ±0.54 | 72.31 ±0.28 | 65.69 ±0.50 |
| 12.0 | 87.54 ±1.81 | 77.44 ±0.54 | 75.99 ±0.29 | 68.97 ±0.50 |

n = 3*

**TABLE . 27 COMPARISION OF IN VITRO RELEASE OF VARIOUS GEL FORMUATIONS (FG1, FG2 & FG3)**

| TIME(MIN) | CUMULATIVE % DRUG RELEASE OF GELS ± S.D* | | |
|---|---|---|---|
| | FG1 | FG2 | FG3 |
| 0.25 | -4.03±1.92 | -9.1 ±2.5 | -7.4±2.62 |
| 0.50 | -1.00±1.95 | -11.13 ±2.01 | -4.84±2.37 |
| 0.75 | 1.49±2.68 | -8.35 ±2.49 | -1.86±2.03 |
| 1.0 | 4.48±1.58 | -6.24 ±2.83 | 1.49±1.52 |
| 1.5 | 6.78±3.00 | -2.40 ±2.96 | 5.32±1.90 |
| 2.0 | 10.01±1.75 | 0.31 ±2.41 | 7.69±1.85 |
| 2.5 | 13.06±2.04 | 2.64 ±2.50 | 10.57±2.49 |
| 3.0 | 16.90±2.13 | 5.34 ±1.95 | 13.16±2.99 |
| 3.5 | 20.98±2.94 | 8.57 ±1.77 | 17.51±3.56 |
| 4.0 | 25.23±2.80 | 10.71 ±2.34 | 20.39±3.83 |
| 4.5 | 29.64±2.83 | 14.17 ±1.96 | 23.79±3.50 |
| 5.0 | 33.81±2.20 | 17.36 ±2.05 | 27.32±3.06 |
| 5.5 | 37.29±2.78 | 19.85 ±2.85 | 30.55±3.17 |
| 6.0 | 41.32±2.30 | 22.83 ±3.37 | 34.72±3.43 |
| 6.5 | 45.83±3.48 | 25.92 ±3.52 | 38.61±3.96 |
| 7.0 | 50.10±3.60 | 29.53 ±3.72 | 43.46±2.18 |
| 7.5 | 54.08±3.06 | 34.09 ±3.38 | 47.23±3.75 |
| 8.0 | 57.74±3.28 | 37.57 ±3.75 | 50.70±2.87 |
| 8.5 | 62.32±2.90 | 41.15 ±2.51 | 55.89±2.98 |
| 9.0 | 67.03±3.03 | 45.26 ±2.10 | 59.60±2.54 |
| 9.5 | 71.85±3.31 | 49.07 ±2.85 | 63.81±2.77 |
| 10.0 | 76.39±3.28 | 53.82 ±2.73 | 68.12±2.85 |
| 10.5 | 81.45±2.98 | 57.88 ±3.56 | 72.54±2.30 |
| 11.0 | 87.04±2.75 | 63.29 ±2.43 | 77.05±2.17 |
| 11.5 | 91.13±3.34 | 66.38 ±3.38 | 81.68±2.15 |
| 12.0 | 95.68±3.53 | 69.92 ±2.53 | 85.57±2.77 |

n = 3*

**TABLE . 18 COMPARISON OF DRUG CONTENT, % ENTRAPPMENT EFFICIENCY & IN VITRO RELEASE PROFILE OF $6^{th}$ & $12^{th}$ HOURS IN DIFFERENT NON-IONIC SURFACTANT CONCENTRATIONS**

| F:NO | DRUG %CONTENT | %ENTRAPPMENT EFFICIENCY | IN VITRO RELEASE | |
|---|---|---|---|---|
| | | | $6^{TH}$HOUR | $12^{TH}$ HOUR |
| F1 | 95.0 | 75.86 | 43.02% | 95.63% |
| F2 | 95.7 | 76.38 | 38.78% | 87.56% |
| F3 | 95.5 | 77.13 | 36.90% | 79.30% |
| F4 | 96.95 | 79.26 | 37.10% | 72.70% |
| F5 | 95.5 | 84.38 | 42.44% | 77.46% |
| F6 | 95.7 | 86.69 | 30.49% | 71.27% |
| F7 | 95.0 | 87.06 | 25.11% | 63.27% |
| F8 | 96.95 | 89.91 | 22.59% | 57.19% |
| F9 | 95.5 | 66.75 | 46.5% | 94.4% |
| F10 | 96.95 | 68.48 | 49.2% | 90.9% |
| F11 | 95.0 | 69.51 | 37.80% | 85.70% |
| F12 | 95.7 | 70.27 | 36.50% | 74.30% |
| F13 | 96.95 | 70.13 | 47.93% | 97.80% |
| F14 | 95.0 | 71.14 | 39.99% | 86.90% |
| F15 | 95.5 | 72.31 | 37.7% | 80.00% |
| F16 | 95.7 | 73.77 | 26.7% | 61.50% |
| F17 | 95.7 | 74.03 | 44.3% | 98.00% |
| F18 | 95.3 | 75.37 | 35.8% | 84.80% |
| F19 | 95.5 | 77.28 | 37.3% | 82.15% |
| F20 | 95.0 | 79.23 | 37.5% | 67.40% |
| F21 | 96.95 | 86.69 | 41.43% | 90.20% |
| F22 | 95.0 | 87.44 | 37.60% | 86.10% |
| F23 | 95.7 | 88.72 | 37.30% | 81.40% |
| F24 | 95.5 | 89.48 | 32.70% | 76.00% |
| F25 | 95.7 | 67.65 | 40.77% | 89.50% |
| F26 | 95.0 | 69.51 | 40.63% | 85.81% |
| F27 | 96.95 | 70.55 | 33.09% | 77.58% |
| F28 | 95.5 | 71.24 | 37.08% | 72.33% |
| F29 | 95.7 | 88.19 | 44.94% | 87.54% |
| F30 | 95.0 | 89.21 | 31.01% | 77.44% |
| F31 | 95.5 | 90.25 | 38.47% | 75.99% |
| F32 | 96.95 | 92.05 | 28.84% | 68.97% |

**TABLE. 19 DETERMINATION OF ORDER OF RELEASE OF LORNOXICAM FROM NIOSOMAL FORMULATIONS**

| Formulation | Higuchi | | Korsemeyer-Peppas | | Zero order | | First order | | Hixon-Crowell | | Release Mechanism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^2$ | $K_H$ ($mg^{-h1/2}$) | $r^2$ | n | $r^2$ | $K_0$ (% mg/h) | $r^2$ | $K_1$ ($h^{-1}$) | $r^2$ | Slope (n) | |
| F1 | 0.924 | 29.31 | 0.958 | 0.765 | 0.987 | 7.240 | 0.809 | -0.084 | 0.898 | -0.206 | HIGUCHI |
| F2 | 0.922 | 27.30 | 0.972 | 0.855 | 0.988 | 6.755 | 0.881 | -0.061 | 0.931 | -0.167 | NFD |
| F3 | 0.945 | 25.10 | 0.988 | 0.861 | 0.995 | 6.154 | 0.930 | -0.049 | 0.961 | -0.140 | NFD |
| F4 | 0.957 | 24.85 | 0.996 | 0.983 | 0.988 | 6.062 | 0.969 | -0.044 | 0.985 | -0.130 | NFD |
| F5 | 0.967 | 23.69 | 0.983 | 0.681 | 0.998 | 5.751 | 0.966 | -0.047 | 0.985 | -0.135 | NFD |
| F6 | 0.908 | 20.46 | 0.963 | 0.782 | 0.980 | 5.079 | 0.914 | -0.036 | 0.942 | -0.108 | NFD |
| F7 | 0.913 | 20.13 | 0.977 | 0.936 | 0.984 | 4.993 | 0.939 | -0.032 | 0.957 | -0.101 | NFD |
| F8 | 0.913 | 18.54 | 0.999 | 1.052 | 0.999 | 4.602 | 0.95 | -0.028 | 0.964 | -0.089 | SUPER CASE II |

**TABLE. 20 DETERMINATION OF ORDER OF RELEASE OF LORNOXICAM FROM NIOSOMAL FORMULATIONS**

| Formulation | Higuchi | | Korsemeyer-Peppas | | Zero order | | First order | | Hixon-Crowell | | Release Mechanism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^2$ | $K_H$ ($mg^{-h1/2}$) | $r^2$ | n | $r^2$ | $K_0$ (% mg/h) | $r^2$ | $K_1$ ($h^{-1}$) | $r^2$ | Slope (n) | |
| F9 | 0.944 | 30.12 | 0.986 | 0.854 | 0.996 | 7.391 | 0.854 | -0.080 | 0.930 | -0.203 | HIGUCHI |
| F10 | 0.965 | 27.91 | 0.986 | 0.704 | 0.993 | 6.765 | 0.894 | -0.07 | 0.949 | -0.181 | HIGUCHI |
| F11 | 0.932 | 27.66 | 0.984 | 0.907 | 0.993 | 6.820 | 0.906 | -0.060 | 0.948 | -0.166 | NFD |
| F12 | 0.953 | 24.25 | 0.992 | 0.877 | 0.998 | 5.929 | 0.959 | -0.045 | 0.979 | -0.131 | NFD |
| F13 | 0.951 | 31.94 | 0.995 | 0.933 | 0.998 | 7.817 | 0.789 | -0.093 | 0.914 | -0.223 | HIGUCHI |
| F14 | 0.937 | 29.06 | 0.988 | 0.974 | 0.995 | 7.154 | 0.912 | -0.064 | 0.954 | -0.176 | NFD |
| F15 | 0.930 | 27.37 | 0.966 | 0.942 | 0.991 | 6.751 | 0.928 | -0.055 | 0.958 | -0.158 | NFD |
| F16 | 0.928 | 20.29 | 0.988 | 1.007 | 0.992 | 5.060 | 0.957 | -0.032 | 0.972 | -0.101 | NFD |

**TABLE. 21 DETERMINATION OF ORDER OF RELEASE OF LORNOXICAM FROM NIOSOMAL FORMULATIONS**

| Formulation | Higuchi | | Korsemeyer-Peppas | | Zero order | | First order | | Hixon-Crowell | | Release mechanism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^2$ | $K_H$ ($mg^{-h1/2}$) | $r^2$ | n | $r^2$ | $K_0$ (% mg/h) | $r^2$ | $K_1$ ($h^{-1}$) | $r^2$ | Slope (n) | |
| F17 | 0.929 | 30.94 | 0.965 | 0.830 | 0.990 | 7.631 | 0.763 | -0.096 | 0.887 | -0.224 | HIGUCHI |
| F18 | 0.928 | 27.98 | 0.986 | 0.967 | 0.991 | 6.909 | 0.909 | -0.059 | 0.949 | -0.165 | NFD |
| F19 | 0.932 | 27.68 | 0.987 | 1.009 | 0.994 | 6.829 | 0.925 | -0.056 | 0.958 | -0.159 | NFD |
| F20 | 0.971 | 23.71 | 0.995 | 0.984 | 0.996 | 5.739 | 0.987 | -0.04 | 0.995 | -0.120 | NFD |
| F21 | 0.927 | 26.39 | 0.969 | 0.756 | 0.988 | 6.511 | 0.855 | -0.062 | 0.920 | -0.166 | HIGUCHI |
| F22 | 0.931 | 27.89 | 0.984 | 0.924 | 0.993 | 6.881 | 0.905 | -0.061 | 0.948 | -0.168 | NFD |
| F23 | 0.944 | 27.33 | 0.990 | 0.987 | 0.997 | 6.712 | 0.936 | -0.054 | 0.967 | -0.155 | NFD |
| F24 | 0.932 | 25.94 | 0.990 | 1.060 | 0.993 | 6.401 | 0.937 | -0.048 | 0.963 | -0.141 | SUPER CASE II |

**TABLE. 22 DETERMINATION OF ORDER OF RELEASE OF LORNOXICAM FROM NIOSOMAL FORMULATIONS**

| Formulation | Higuchi | | Korsemeyer-Peppas | | Zero order | | First order | | Hixon-Crowell | | Release mechanism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^2$ | $K_H$ ($mg^{-h1/2}$) | $r^2$ | n | $r^2$ | $K_0$ (% mg/h) | $r^2$ | $K_1$ ($h^{-1}$) | $r^2$ | Slope (n) | |
| F25 | 0.939 | 29.17 | 0.991 | 0.943 | 0.995 | 7.175 | 0.887 | -0.067 | 0.993 | -0.082 | NFD |
| F26 | 0.939 | 29.13 | 0.991 | 1.008 | 0.995 | 7.167 | 0.920 | -0.063 | 0.958 | -0.175 | SUPER CASE II |
| F27 | 0.919 | 26.35 | 0.989 | 1.093 | 0.990 | 6.532 | 0.928 | -0.050 | 0.956 | -0.146 | SUPER CASE II |
| F28 | 0.958 | 24.81 | 0.996 | 0.979 | 0.998 | 6.052 | 0.970 | -0.044 | 0.985 | -0.131 | NFD |
| F29 | 0.948 | 28.39 | 0.980 | 0.847 | 0.997 | 6.959 | 0.928 | -0.065 | 0.965 | -0.176 | NFD |
| F30 | 0.884 | 23.72 | 0.954 | 0.908 | 0.973 | 5.944 | 0.894 | -0.045 | 0.927 | -0.133 | NFD |
| F31 | 0.941 | 24.39 | 0.978 | 0.882 | 0.996 | 5.997 | 0.950 | -0.046 | 0.972 | -0.134 | NFD |
| F32 | 0.909 | 22.49 | 0.973 | 1.006 | 0.985 | 5.595 | 0.937 | -0.038 | 0.957 | -0.117 | SUPER CASE II |

**TABLE. 28 DETERMINATION OF ORDER OF RELEASE OF LORNOXICAM FROM NIOSOMALGEL FORMULATIONS**

| Formulation | Higuchi | | Korsemeyer-Peppas | | Zero order | | First order | | Hixon-Crowell | | Release mechanism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $r^2$ | $K_H$ ($mg^{-h1/2}$) | $r^2$ | n | $r^2$ | $K_0$ (% mg/h) | $r^2$ | $K_1$ ($h^{-1}$) | $r^2$ | Slope (n) | |
| G1 | 0.9957 | 33.89 | 0.9863 | 1.347 | 0.9974 | 8.3158 | 0.8293 | -0.0866 | 0.7491 | 0.362 | HIGUCHI |
| G2 | 0.9805 | 27.46 | 0.9719 | 2.1166 | 0.9925 | 6.7768 | 0.9377 | -0.0438 | 0.8143 | 0.5254 | HIGUCHI |
| G3 | 0.9964 | 31.33 | 0.9835 | 1.462 | 0.9966 | 7.6827 | 0.9119 | -0.0629 | 0.7293 | 0.4107 | HIGUCHI |

## TABLE. 23 COMPOSITION OF GEL

| SL. NO | INGREDIENTS | FOR 100 G |
|---|---|---|
| 1. | Carbopol 940 | 1g |
| 2. | Triethanolamine | 0.165 ml |
| 3. | Water | To 100 ml |
| 4. | Drug(Lornoxicam) | 20 mg |

## TABLE. 24 DRUG CONTENT OF GEL FORMULATION

| SL. NO | FORMULATION | DRUG CONTENT |
|---|---|---|
| 1. | G1 | 95.4% |
| 2. | G2 | 96.2% |
| 3 | G3 | 95.0% |

## TABLE. 25 $P^H$ VALUES

| S. No | FG1 | FG2 | FG3 |
|---|---|---|---|
| 1 | 6.8 | 6.8 | 6.8 |
| 2 | 6.8 | 6.8 | 6.8 |
| 3 | 6.8 | 6.8 | 6.8 |

**TABLE. 26**

**BROOKFIELD DV II+LV VISCOMETER**

**SPINDLE - 64 Temperatures: 28° C**

| S.NO | RPM | VISCOSITY IN CPS | |
|---|---|---|---|
| | | FG2 | FG3 |
| 1 | 0.1 | 20000 | 19500 |
| 2 | 0.5 | 5210 | 3100 |
| 3 | 1.0 | 3900 | 1510 |
| 4 | 5.0 | 1180 | 670 |
| 5 | 10.0 | 748 | 340 |
| 6 | 20.0 | 500 | 200 |
| 7 | 50.0 | 270 | 114 |
| 8 | 100 | 175 | 72 |

## TABLE. 29

## % INHIBITION OF PAW OEDEMA

| S.NO | GROUP | TREATMENT | '1' h | '2' h | '3' h | '4' h | '5' h | '6' h | '24' h |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Group –I | FG1 | 28.40 | 28.64 | 32.58 | 33.90 | 35.08 | 41.76 | 39.64 |
| 2 | Group –II | FG2 | 11.0 | 12.59 | 13.74 | 14.96 | 18.51 | 19.54 | 22.36 |
| 3 | Group-III | FG3 | 26.19 | 27.73 | 32.58 | 34.46 | 38.01 | 39.50 | 40.11 |

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